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A SYSTEM OF ANALYSIS FOR PLANT TISSUE BY USE OF PLANT JUICE[1]

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The utilization of expressed plant juice for determining the chemical constituents of a plant is becoming increasingly important. It has the advantage of presenting the products of metabolism in a state more similar to that found in living tissue than any of the more commonly used extraction methods. Conditions of state and equilibrium, however, can probably never be identical for juice within and without the cell, for at the moment of cell rupture, the conditions that control living cellular activity are changed. To illustrate, if living leaf tissue is placed in an atmosphere of carbon dioxide, the juice becomes more basic (**8**), but if the cell liquid is expressed, and carbon dioxide bubbled through it, the juice becomes more acid.

The first important studies of expressed juice were for the investigation of physical constants such as density, refractive index, freezing point, etc. This work has been summarized by SASTRI and SREENIVASAYA (**21**). Recently, attention has been paid to the inorganic and more particularly to the organic fractions, of plant juice including the sugars and nitrogen fractions. This paper is presented to describe methods for the determinations of a portion of the inorganic and organic constituents in plant juice extracts, and calculation of the results on a fresh weight basis.

General methods

Before a representative sample of juice can be obtained from the entire tissue studied, the plant material must be treated in some manner so that the contents of all cells are represented. For example, unrepresentative juice samples are obtained by pressure alone, if the freezing point lowering is used as a criterion. This uncertainty may be obviated, to a large extent, by treating the sample in various ways: by grinding, freezing with solid carbon dioxide, liquid air, or eutectic mixtures; by vapors of volatile liquids such as ether or chloroform; or by autoclaving (**1, 6, 7, 11, 12, 21**). The work of SAYRE and MORRIS (**22**) with corn indicates that grinding is as effective in breaking cell walls as freezing. This is advantageous for there

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is some indication that during the freezing of tomato leaves the sucrose-reducing sugar equilibrium is shifted toward an increase in reducing sugars. Substances such as nitrates, inorganic phosphates, and total sugars are expressed in like amounts in successive fractions, after a preliminary grinding of corn tissue (**23**). Treatment of plant material by autoclaving at five pounds per square inch for 15 minutes gives results comparable to the freezing technique (**7**) and similarly causes the precipitation of proteins.

For the data presented in this paper, the ordinary Nixtamal mill was used for grinding leaf samples. When samples were frozen, they were first cut and wrapped tightly in a cheese-cloth bag, exposed to carbon dioxide snow as it was formed at the end of a copper tube attached to the tank of liquid carbon dioxide. This gave rapid and complete freezing of a 100-gram sample in less than a minute. Unless otherwise stated, all frozen samples used in this study were so treated.

It is evident that the preliminary treatment of plant tissue depends upon the substances to be determined. Grinding is apparently adequate for all circumstances and its only evident disadvantage, in comparison with freezing or autoclaving, is that the resulting juice contains large quantities of debris and protein. Where proteins must be removed, their partial elimination by freezing or autoclaving is helpful.

Determination of physical constants

Sayre and Morris (**23**) have proposed a formula[2] whereby the various plant constituents can be determined by analysis of expressed juice and the results calculated to the fresh-weight basis. The values obtained were comparable to those secured by an alcohol extraction. Using this equation as a basis, it was advisable to examine the methods available for the determination of specific gravity, total solids of plant juice, and the total moisture of the leaf material since these values are necessary for the calculation.

For the determination of specific gravity three methods are available: the Westphal balance, hydrometer, and pycnometer. In a series of 20 juice samples of beet and tomato leaves using the methods mentioned, the average values obtained were 1.036, 1.025, 1.022, respectively. In all instances the agreement between these methods was sufficiently accurate so that any one could be used. The hydrometer method, however, is by far the most rapid and convenient.

Gortner (**13**) has demonstrated the possibility of determining total solids and subsequently the moisture content of plant juice with the refractometer. This method has been shown (**17**) to give high results in comparison with oven drying for juice samples of soybean, corn, wheat, and oats. Sayre and Morris (**23**) found that for corn it was necessary to correct the total solids determined by refractometer when leaf juice was used, but no correction was needed for juice from stalk tissue.

[2] $$\text{Percentage of sugar in tissue} = \frac{\dfrac{\text{Gm. of sugar in 100 ml. sap}}{\text{Gm. of moisture in 100 ml. sap}} \times \text{Total moisture of tissue in gm.}}{}$$

In the present study a comparison has been made between the toluene distillation method (**3**) for moisture in plant juice and the refractometer method. The refractometer was adjusted to give the theoretical value for water at 20° C. The scale reading gives the refractive index of the juice directly, and SCHÖNROCK's (**2**) table was used to translate refractive index values to total solids. The percentage of juice moisture is the difference between the percentage of total solids and 100. In table I, the results by the above mentioned methods are expressed in terms of moisture content rather than total solids.

The estimation of total moisture in leaf tissue was also made by oven drying at 100° C. for 24 hours. In order to be assured of the accuracy of this method it was examined in two ways. First, samples were prepared by cutting the leaves into small sections and weighing aliquots into aluminum dishes. One series was frozen with carbon dioxide snow, the other was untreated. The freezing technique was introduced to make certain that the cell walls were broken so that all leaf moisture could be removed. Using juice from tomato leaves and beet leaves and beet petioles the agreement between frozen and non-frozen samples was within 0.5 per cent. Similarly, with the same tissues the average of six determinations gave the following results: oven drying, 86.88 per cent.; toluene distillation, 86.75 per cent. moisture.

CHEMICAL METHODS

In a study of the nitrogen metabolism of plants certain fractions are usually determined; nitrate, ammonia, alpha-amino, amide, and total nitrogen. Carbohydrate analyses usually include sucrose and reducing sugars, which in turn may be divided into glucose, fructose, and non-sugar reducing substances.

NITRATE NITROGEN.—BURRELL and PHILLIPS (**4**) for alcohol extracts and FREAR (**10**) for plant juice have shown that clarification of plant juice is possible with silver sulphate, copper sulphate, and a mixture of calcium

TABLE I

PERCENTAGE OF MOISTURE IN JUICE FROM BEET LEAVES AS DETERMINED BY REFRACTOMETER AND TOLUENE DISTILLATION METHODS

METHOD	MOISTURE, PERCENTAGE								
	1	2	3	4	5	6	7	8	AVERAGE
	%	%	%	%	%	%	%	%	%
Refractometer	91.93	89.93	93.20	93.90	95.20	93.90	94.50	95.20	93.46
Toluene distillation	92.00	91.40	92.90	94.50	95.00	93.00	93.40	93.90	93.26

hydroxide and magnesium carbonate or calcium hydroxide alone. BURRELL states that it was necessary to make a preliminary treatment with sodium peroxide for alcoholic extracts. FREAR did not try this for plant juice but secured satisfactory results. Nitrate is then determined in the clarified juice by the phenol-disulphonic acid method (**15**).

AMMONIA NITROGEN.—While studying the various methods for ammonia, SCHLENKER (**24**) found that when either 52 per cent. potassium carbonate or heavy magnesium oxide was added to plant juice and the sample aspirated, there was a rapid evolution of ammonia during the first two hours but that with continued aeration ammonia continued to be given off in small amounts over a long period of time. A more satisfactory procedure for the determination of ammonia was devised by shaking juice with 2.0 ml. of sodium permutit (FOLIN) for 5–7 minutes to adsorb the ammonia, then removing the juice by washing, thus eliminating the glutamine and asparagine. Ammonia is freed from the permutit by sodium hydroxide and aspirated as usual the Van Slyke-Cullen aspiration block.

PUCHER (**19**) later reported that the amide nitrogen of glutamine is hydrolyzed quite readily, more easily than that of asparagine. Therefore, it is to be expected that when glutamine is present in a plant juice which is treated with an alkali such as potassium carbonate and aspirated, some of the glutamine amide nitrogen will be included with that of the preformed ammonia. It can be shown that asparagine is only slightly hydrolyzed by potassium carbonate (**27**).

GERDEL (**11**) modified the SESSION and SHIVE method for the determination of ammonia and nitrate nitrogen; but, as this method is based on the release of ammonia directly from plant material in the presence of alkali, high results are obtained.

ALPHA-AMINO NITROGEN.—The VAN SLYKE (**30**) method for the estimation of amino nitrogen has been examined by STUART (**29**) who found that high results are obtained when using water or alcoholic plant extracts. A number of substances such as ammonia, dihydroxy phenols, and tannic acid were found to yield nitrogen with nitrous acid. Several means were used to eliminate these error-producing substances. The lowest amino nitrogen values were obtained after adding calcium oxide and using vacuum aspiration at 40–45° C. for one hour with subsequent removal of excess lime. It was further demonstrated that amino acid and asparagine nitrogen could be recovered quantitatively after this treatment. It is probable, however, that a portion, if not all, of the glutamine present was destroyed during the vacuum distillation.

Ninhydrin, a specific reagent for alpha-amino acids, has been used to determine the amino nitrogen in plant extracts (**28**). If the results using this reagent are taken as true values, the figures obtained by the nitrous acid method are too high even after treatment with lime. The reliability of the nitrous acid method depends on the extent to which interfering substances can be removed and apparently lime treatment is insufficient. In the use of ninhydrin it is necessary to precipitate proteins and desirable to remove preformed ammonia. Because of specificity, methods using ninhydrin will undoubtedly give results that are close to the true alpha-amino nitrogen content of plant extracts.

GLUTAMINE AND ASPARAGINE AMIDE NITROGEN.—These amides are usually determined together by hydrolysis with normal sulphuric acid but CHIBNALL

and WESTALL (**5**) and VICKERY *et al.* (**31**), by adjusting the hydrogen-ion concentration of the solution were able to hydrolyze glutamine in the presence of asparagine, with the inclusion of only a small amount of the latter. At pH 6.0–6.5 between 98.5 and 99.0 per cent. of glutamine amide nitrogen is hydrolyzed after two hours at 100° C. In the presence of normal sulphuric acid for three hours both amides are completely hydrolyzed. Asparagine amide nitrogen is obtained by difference on the assumption that only these two amides are present. This procedure has been applied to plant juice (**27**).

PUCHER (**20**) developed a method for the estimation of glutamine which supplements the method just described. This was based on the ethyl-acetate extraction of the pyrrolidone-carboxylic acid formed during the nearly neutral hydrolysis of glutamine. After extraction the carboxylic acid was hydrolyzed to glutamic acid. By determining amino nitrogen before and after the second hydrolysis the amount of glutamine present was calculated.

ORCUTT and WILSON (**18**) advocate the use of 20 per cent. sodium bisulphite to avoid humin formation which occurs during the determination of total amide by 10 per cent. sulphuric acid. It is probable that bisulphite may be used on juice samples to avoid the necessity of precipitating the protein fraction. Determinations of total amides were made on protein-free and protein-containing juice treated with 10 per cent. sulphuric acid and 20 per cent. sodium bisulphite (table II).

It is evident that bisulphite is sufficiently active to hydrolyze plant juice proteins so that it is necessary to remove these proteins before using this reagent; the same holds true for sulphuric acid.

DETERMINATION OF SUGARS.—The colorimetric method of FOLIN (**9**) using an alkaline copper tartrate oxidizing solution gives satisfactory results with plant juice (**25, 26**). In the preparation of the tissue extract, freezing is not always a satisfactory method, for in some instances during this process there is a shift in the sucrose-reducing sugar equilibrium with an increase in the reducing sugars. In tomato leaf reducing sugar increased 0.13–1.70 mg. per ml. of juice. This difference is not due to sampling errors for, when a sample of plant juice expressed from ground beet leaf was divided into two portions and only one portion frozen, its reducing sugar content was increased by 0.39 mg. per ml. as compared with the unfrozen juice sample.

However, storage of corn leaves or juice from the same samples of corn leaves in a cold storage room at approximately 0° C. for a period of over a month had no effect on the sucrose-reducing sugar equilibrium (unpublished data).

Procedure for analysis

STANDARDS

The proposed methods for nitrogen compounds, with the exception of nitrate, are all based on the colorimetric determination of ammonia by Nesslerization after alkaline aspiration. The sugar methods depend on the reduction of phospho-molybdate reagent by cuprous oxide and comparison

of the resulting blue solution with a standard in a colorimeter. No one set of working standards can be recommended because of the great variations in concentrations of plant constituents, depending on a number of factors. The dilute stock standards generally used were as follows: 40 mg. ammonia nitrogen per liter from ammonium sulphate, 200 mg. glucose (Bureau of Standards) per liter, and 10 mg. nitrate nitrogen per liter from potassium nitrate.

TABLE II

TOTAL AMIDE-NITROGEN IN CLARIFIED AND UNCLARIFIED BEET JUICE

TREATMENT	AMIDE-NITROGEN PER MILLILITER OF PLANT JUICE	
	10% H_2SO_4	20% $NaHSO_3$
	mg.	*mg.*
Unclarified	0.0167	0.0117
Clarified*	0.0020	0.0050
Unclarified	0.0696	0.0486
Clarified	0.0150	0.0147

* Clarified by 20 per cent. tannic acid solution. Preformed ammonia removed from all samples.

Ammonia is aspirated into approximately 0.01 normal sulphuric acid and determined in all cases by Nesslerization. After aeration the samples are transferred to 50-ml. volumetric flasks and made up to within 3 ml. of the mark, mixed, and Nessler's reagent added. Aliquots are so chosen that the ammonia nitrogen concentration in the final dilution is never greater than 0.0016–0.004 mg. per ml.

If a photoelectric colorimeter is available the use of transmission-concentration curves dismisses the need of working standards.

PREPARATION OF SAMPLE

Grind the sample in a Nixtamal mill or freeze with solid carbon dioxide and allow to thaw. Express the juice by means of a hydraulic press and centrifuge the resulting juice. Determine total solids by the Abbé refractometer and specific gravity with a hydrometer. Dry a fresh leaf sample in an oven at 100° C. overnight for total moisture.

TOTAL SOLUBLE NITROGEN

Filter plant juice through asbestos to remove all suspended material. Pipette a suitable aliquot and 1 ml. of an acid mixture[3] into a large Pyrex test tube and add a glass bead. To insure the reduction of nitrate add a small amount of zinc, and heat if necessary. Heat over a small flame to complete digestion. Place the tubes in an aspiration block, make the usual connections, add 1 ml. of toluene and sufficient sodium hydroxide to make the mixture alkaline, and aspirate.

For soluble inorganic nitrogen, an alternative procedure is available (**11**).

[3] 5 ml. 5 per cent. $CuSO_4$, 30 ml. 85 per cent. H_3PO_4, 10 ml. concentrated H_2SO_4. This combination is mixed 1:1 with water and used as suggested in the text.

Place an aliquot in the tubes in the aspiration block, add Devarda's alloy, and then alkali, causing reduction of nitrates during the alkaline rather than the acid stage. This requires a long aspiration period.

Nitrate nitrogen (10)

Pipette a juice aliquot, 1 or 2 ml., in a 100-ml. volumetric flask containing 50 ml. water and 1 or 2 ml. of 5 per cent. $CuSO_4 \cdot 7\ H_2O$, mix and add a sufficient volume of saturated Ag_2SO_4 solution to precipitate chlorides. Finally add enough solid $Ca(OH)_2$ $MgCO_3$, 4 + 10 mixture, to precipitate excess copper. Make to volume, mix, and filter through a dry paper. Evaporate a 25-ml. aliquot of the sample to dryness. Prepare a standard by evaporating 10 ml. of a solution containing 10 p.p.m. of N from KNO_3 to dryness. Add 1 ml. of phenoldisulphonic acid solution and allow to react with the nitrate. Then add water followed by sodium hydroxide until the characteristic yellow color appears. Dilute the standard and sample to 100 ml. and compare in a colorimeter.

Ammonia nitrogen (24)

Add 1 to 5 ml. of juice to 2.5 ml. of permutit (Folin) in an ammonia aspiration tube and shake at least three minutes. Remove extraneous juice by washing with water and decanting a sufficient number of times to remove all color. To the permutit add approximately 5 ml. of water and 1 ml. of toluene. Place the tubes in an aspiration block and add 4 ml. of 10 per cent. NaOH; connect the apparatus and aspirate 2.5 hours into approximately 0.01 N H_2SO_4. To reduce the time, the reaction tubes may be immersed in a water bath at 55–60° C. and aspirated 30 minutes. If ammonia is determined by Nessler's reagent the normality of the acid need only be approximate.

Clarification of juice sample for the determination of amide and alpha-amino nitrogen

Treat 10 ml. of juice with 5 ml. of 5 per cent. sodium tungstate in 0.33 N H_2SO_4. After proteins and colored substances have been precipitated, centrifuge to remove suspended material. Pour a sample of the supernatant liquid into a test tube and shake with a sufficient amount of permutit to remove preformed ammonia.

Amide nitrogen (27)

Pipette 5 ml. or less of the protein- and ammonia-free juice into an ammonia aspiration tube; add 1 ml. 6 N H_2SO_4 and sufficient water, if necessary, to make the total volume 6 ml. At the same time place 10 ml. of phosphate buffer pH 6.0–6.5 in a second ammonia tube and add a suitable aliquot of the protein and ammonia-free juice. Place both tubes, fitted with stoppers containing capillary tubes, in a boiling water bath. Hydrolyze the first tube three hours for total amide nitrogen, and the second tube two hours for glutamine amide nitrogen. At the end of the period, cool the tubes, place in an aspiration block, and add 1 ml. of toluene. For total

amides add 3–4 ml. of a 10 per cent. NaOH solution. To make the glutamine tube alkaline add 2 ml. of 52 per cent. K_2CO_3. Aspirate and determine ammonium nitrogen as above. The difference between total amide nitrogen and glutamine amide nitrogen is considered to be asparagine amide nitrogen.

Amino nitrogen (28)

Transfer a protein- and ammonia-free sample containing not more than 0.20 mg. alpha-amino nitrogen into an ammonia tube, add 1 ml. phosphoric acid (2 ml. 85 per cent. H_3PO_4 + 1 ml. H_2O) and 0.5 ml. ninhydrin solution (15 mg.). Close the tube with a stopper containing a capillary glass tube 5 cm. long. Place in a boiling water bath for 45 minutes. Remove to an aspiration block, add 4 ml. saturated NaOH solution and aspirate 30 minutes at 50–60° C., or two hours at room temperature. Determine ammonia by titration or Nesslerization. In samples where glutamine and asparagine are extremely high due to abnormal environmental conditions, it is advisable to utilize the sample used for the determination of total amides. After the removal of total amide nitrogen as ammonia, adjust the contents of the tube containing the alkaline mixture to pH 1.0 with concentrated phosphoric acid, add 15–30 mg. of ninhydrin, and place the tube in a boiling water bath for 45 minutes. After cooling determine the ammonia by alkaline aspiration as previously described.

Clarification of juice for the sugar determination

Pipette an aliquot of juice, 1 or 2 ml., into a centrifuge tube (15 or 50 ml.) and dilute to 10 ml.; add 1 or 2 ml. saturated neutral lead acetate solution and centrifuge. Add 10 per cent. Na_2HPO_4 solution until the sugar solution is blue to bromthymol blue, dilute to volume, and again centrifuge.

Determination of total sugar (25)

Transfer an aliquot of the clarified plant juice to a Folin sugar tube, add 2 drops of a 0.5 per cent. Wallenstein's invertase scale preparation. After letting stand for two hours at room temperature, make the solution alkaline and add 2 ml. of Folin alkaline copper tartrate (9). Place the tubes in a boiling water bath for 15 minutes, remove and cool, and add the acid molybdate solution for color development; make the solution to volume.

Reducing sugar

Determine this fraction in the lead-free, clarified juice, using a 1- or 2-ml. aliquot, by the Folin alkaline tartrate method mentioned above.

Non-sugar reducing substance (26)

Place 2 ml. of a thoroughly washed 10 per cent. yeast suspension in a 15-ml. centrifuge tube and centrifuge to destroy the suspension. Pour off the supernatant liquid and dry the sides of the tube thoroughly with strips of filter paper. Add an aliquot of clarified juice or fresh juice, about 4 ml., and stir. The tube is placed in a water bath at 37–39° C. and agitated frequently. Centrifuge after 20 minutes and determine residual reducing

power of the sample. This value is used to correct the results for total and free reducing sugars.

Summary

This paper summarizes efforts during a considerable period to select and adapt chemical methods for the analysis of plant juice. Most of the procedures are applicable to other types of plant extracts. The nitrate and sugar methods, however, cannot be used with alcohol or water extracts if a visual colorimeter is used because the precipitating reagents involved do not give the necessary colorless filtrates.

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EFFECT OF SALT CONCENTRATION, KIND OF SALT, AND CLIMATE ON PLANT GROWTH IN SAND CULTURES[1]

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(WITH NINE FIGURES)

Introduction

Many western farmers believe that soil alkali is more detrimental to crop growth during the hot weather than during the cooler seasons of the year. Other agricultural workers have noted that damage to crops due to alkali (soil salinity) is more serious in the hot interior valleys than along the coast where the climate is more moderable. It is not definitely known whether or not soil salinity concentrations are comparable between different seasons of the year or between respective areas. Yet, information on this question is pertinent to the development of management practices which would aid in ameliorating soil salinity conditions at different seasons of the year and under different climatic conditions.

In order to obtain definite information on this question, the Regional Salinity Laboratory planned a series of experiments to determine the tolerance of crop plants to the salts commonly found in irrigation waters and soils, and to what extent climate modified these effects. This paper presents a resumé of the more pertinent results from these experiments conducted during 1939, 1940, and 1941.

Trelease and Livingston (**10**) were among the earliest authors to find that climatic conditions modified the crop growth obtained with the same culture solutions and to suggest that studies should be made on the effect of climate in modifying plant responses.

In 1938 Ahi and Powers (**1**) grew salt grass, alfalfa, and strawberry clover in the same culture solutions in greenhouses held at 55° and 75° F. They found germination and growth to be poorer at the higher temperature.

Hayward and Long (**6**), and Wall and Hartman (**11**) have also published results indicating that climatic factors modify the action of salt on plants.

The amount of salt which plants can tolerate in sand cultures under normal climatic conditions has been reported by many authors, and the reader is referred to recent reviews by Eaton (**5**), and Hayward and Long (**6**). In general, salt tolerant plants can continue growth in nutrient solution at concentrations exceeding 6 atmospheres, while salt sensitive plants may succumb at solution concentrations below 2.5 atmospheres.

Experimentation

The work was done during the summers of 1939, 1940, and 1941 in large

[1] Contribution from the U. S. Regional Salinity Laboratory, Bureau of Plant Industry, Riverside, California, in cooperation with the eleven Western states and the Territory of Hawaii.

outdoor sand cultures at three locations, each having a distinctly different climate. These locations were Torrey Pines, near San Diego; Riverside; and Indio, in the Coachella Valley. The location of these places in Southern California is shown in figure 1. In spite of the diversity of climates the three locations were so near each other that the work could be adequately supervised and coordinated from Riverside. The stations were also in the same approximate latitude so that length of day was essentially the same at all three.

The sand culture tanks have been described by EATON (**4**) and each had a growing area of 3.248 square meters and a reservoir of culture solution containing 2400 liters. By means of motor pumps and time clocks the sand bed could be flooded with the culture solution at definite time intervals, thus bathing the plant roots in the culture solution, providing aeration, and insuring against accumulation of salts on the surface of the sand. The culture

FIG. 1. Outline map of Southern California showing location of Salinity experiments to test effect of climate. These tests were conducted at Riverside, Indio, and Torrey Pines.

solution drained through the sand to the reservoir below. The sand contained 0.2 per cent. magnetite to provide the iron needed by crops as suggested by CHAPMAN (**2**). The composition of the culture solutions used in 1941 is given in table I. They were maintained at about pH 6.5.

The salts used in making up these solutions were carefully selected for low contents of boron and heavy metals.

Growth responses obtained in these culture solutions are believed to be qualitatively characteristic of plant growth in soil solutions of equal salt content, but it should be borne in mind that drainage, aeration, nutrient supply and other growth factors will differ in soils from conditions found in sand culture beds.

EFFECT OF SALT CONCENTRATION

Early investigators, who conducted culture solution experiments to explain the action of alkali on crops in the field, have shown that at high salt concentrations growth is reduced. In figure 2, results of this nature are

TABLE I

COMPOSITION AND CHARACTERISTICS OF CULTURE SOLUTIONS USED IN 1941

TREATMENT	CATIONS PER LITER			ANIONS PER LITER		TOTAL ANIONS* PER LITER	TOTAL SALTS	CONDUCTANCE	OSMOTIC CONCENTRATION
	Na	Ca	Mg	Cl	SO_4				
	mg. eq.	*mg. eq.*	*mg. eq.*	*mg. eq.*	*mg. eq.*	*mg. eq.*	*p.p.m.*	*K* × 10^6 @ 25° C.	*Atmospheres*
Base nutrient	2.6	3.2	2.3	2.5	1.8	10.7	1046	125	0.4
2.4 chloride	36.4	8.4	15.3	54.5	1.8	62.7	3929	649	2.4
4.4 chloride	68.9	13.2	27.8	104.5	1.8	112.7	6697	1137	4.4
2.4 sulphate	58.5	11.8	23.8	2.5	87.8	96.7	6896	753	2.4
4.4 sulphate	121.6	21.5	50.1	2.5	184.8	193.7	13520	1342	4.4
4.4 chloride sulphate mixture	87.1	16.2	34.8	67.5	67.8	141.7	9119	1195	4.4

* All solutions also contained 3.45 mg. eq. of K; 6.0 mg. eq. of NO_3; 0.4 mg. eq. of H_2PO_4; and boron 0.6 p.p.m.; Mn 0.2 p.p.m. These are included in the totals above.

shown for Early Wonder garden beets, Early French forcing carrots, and Weber wax beans grown in culture solutions of varying chloride concentration at Riverside during 1941.

The nutrient concentrations reported in figure 2 are given in terms of atmospheres of osmotic concentration. It is recognized that workers in the field of salinity have used a number of other indices to measure the concentration of salt on a soil or soil solution basis, such as parts per million, milligram equivalents per liter, equivalents per million, conductance[2] of a soil paste, and conductance of a soil extract, in addition to osmotic values of soil solutions or culture solutions. In this paper osmotic concentration

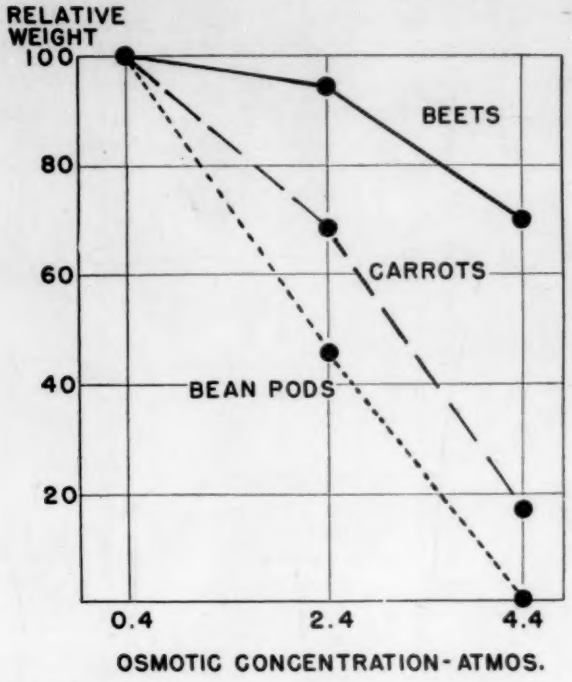

FIG. 2. Differential effect of culture solutions of varying concentration on growth of various plant species.

in atmospheres of the culture solution as determined by freezing point depression is used as our basic index. There seems to be increasing evidence (**4, 5, 8**) that water absorption is related to, or a part of, salinity effects, and osmotic values correlate with such phenomena. Table I gives values for the culture solutions used in 1941 in terms of milligram equivalents per liter, parts per million, conductance, and osmotic concentration.

The results shown in figure 2 are representative of a number of tolerance tests and were obtained at Riverside in the chloride series. The yield of plants grown in the base nutrient solution can be taken as 100 per cent., and

[2] Specific electrical conductances are given in reciprocal ohms and multiplied by 10^5 to avoid decimals. The values are therefore given in terms of $K \times 10^5$ at 25° C.

yields obtained in the 2.4 and 4.4 atmosphere culture solutions plotted as a percentage of the base yield. When this is done the relationship of yield to substrate concentration is nearly linear.

It is clear from the data in figure 2 that in the case of beans the percentage decrease in growth from 0.4 atmospheres to 2.4 atmospheres is equal to the decrease from 2.4 atmospheres to 4.4 atmospheres. The yield of carrot roots shows the same uniform percentage decrease in growth for each additional atmosphere of nutrient concentration. In the case of garden beets the yield at 2.4 atmospheres is greater than the mean of yields at 0.4

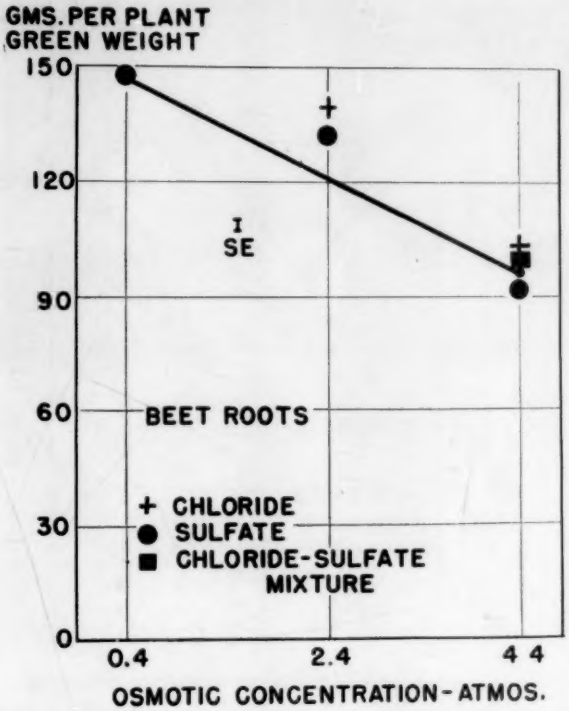

FIG. 3. The relative yield of garden beet roots grown in culture solutions of chloride, sulphate, and mixed chloride-sulphate salts at indicated osmotic concentrations. A measure of the accuracy is given by the length of the line S.E. (Standard error).

and 4.4 atmospheres, but the deviation from this mean is not statistically significant.

The relationship shown between yield and osmotic concentration is almost equally good between yield and conductance of the culture solution. This follows because conductance and osmotic concentrations of the solutions are directly related. This is shown by data in table I. These data show that the ratio of conductance to atmospheres of osmotic concentration lies between 250 and 300, depending on concentration and the nature of the salt present.

Differences in salt tolerance exhibited by various plant species

Field experience has shown that some plants like Atriplex, Russian olive, sugar beets, and cotton have a high tolerance to salts, while others, such as beans and squash, are much less tolerant. Differences in tolerance to salinity exhibited by plant species are shown by the data in figure 2. The slope of the yield line is an index of tolerance, and beans with a steep slope are the least tolerant of the three crops shown. In fact at 4.4 atmospheres no fruit yield was obtained in the case of beans whereas a 68 per cent. yield of beets was harvested at this same concentration. Yield records at River-

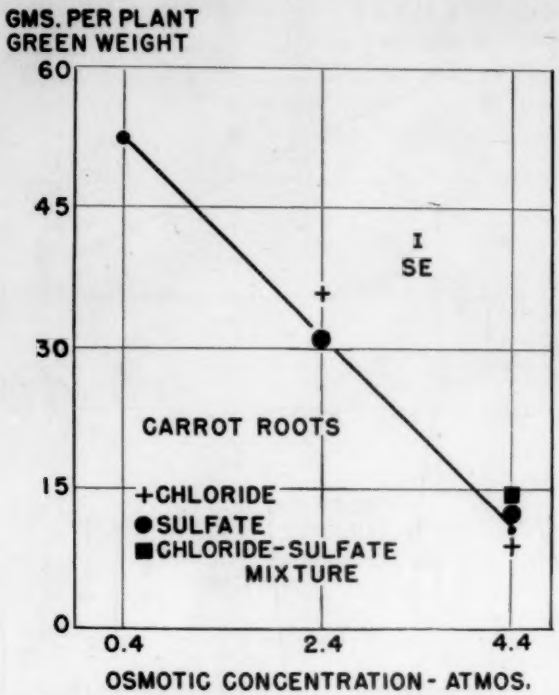

Fig. 4. The relative yield of carrots grown in culture solutions of chloride, sulphate, and mixed chloride-sulphate salts at indicated osmotic concentrations. A measure of the accuracy is given by the length of the line S.E. (Standard error).

side in the presence of chloride solutions suggest the following order for decreasing crop tolerance: Sugar beets, table beets, cotton, alfalfa, cowpeas, tomatoes, milo maize, carrots, squash, onions, and beans.

Effect of anion

The ions most prevalent in saline irrigation waters are sodium, calcium, and magnesium among the cations; and sulphates, chlorides, and bicarbonates among the anions. Almost always these six ions are found in the irrigation water or soil solutions and earlier investigators have studied the systems with a view toward determining if any one or more of the ions was

particularly toxic to plant growth. Such experiments have not been easy to conduct because equal quantities of cations and anions must be present in a culture solution, as well as the necessary plant foods. The action of particular anions has usually been obtained by comparison with equal moles or equivalents of anions in combination with the same cations in the culture medium. The results of experiments in 1939 and 1940 in which equal moles of chlorides and sulphates were compared suggested that comparable re-

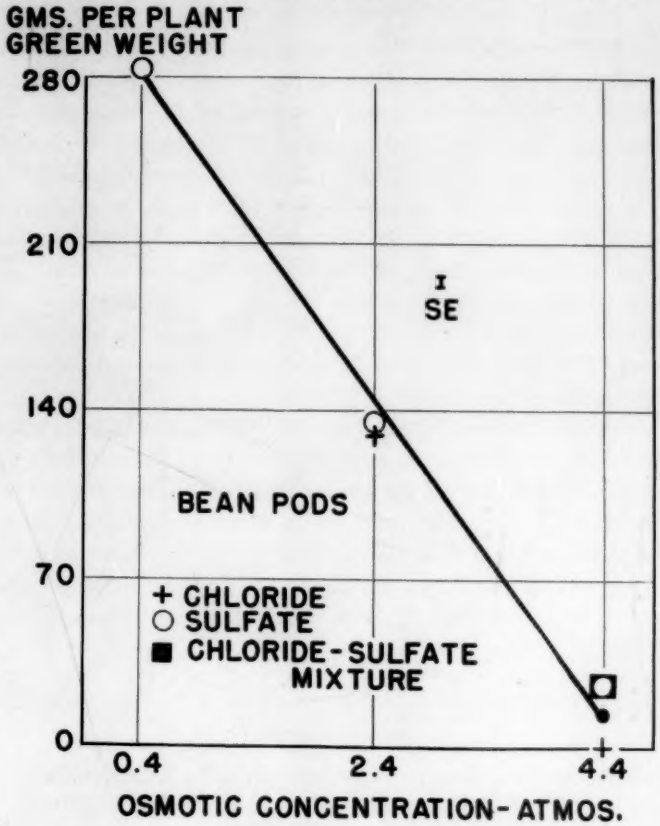

FIG. 5. The relative yield of beans grown in culture solutions of chloride, sulphate, and mixed chloride-sulphate salts at indicated osmotic concentrations. A measure of the accuracy is given by the length of the line S.E. (Standard error).

sponse might be secured if the culture solutions were prepared on an equal osmotic basis. This was done in 1941. The yields obtained on this basis for beets, carrots, and beans are shown in figures 3, 4, and 5. At equal osmotic concentrations the yield of these crops did not differ appreciably.

The data obtained in 1939 and 1940 for alfalfa show that chlorides were more toxic than sulphates at approximately equal osmotic values. This has also been found to be true for peaches (**7**). These results would indicate that for some crops chlorides and sulphates at equal osmotic concentrations

are equally harmful, while with other crops, chlorides are more toxic than sulphates at approximately equal osmotic values. More equivalents of sulphates than of chlorides are required to produce a given freezing point depression or osmotic value. Furthermore, the sulphate ion is somewhat heavier than the chloride ion. These facts explain why plants can withstand far greater amounts of sulphate than chloride when expressed on a parts per million basis.

Effect of cation

During 1939, sand culture tests were conducted which included treatments in which calcium, magnesium, and sodium each predominated. Thus in one treatment 95 equivalents out of a total of 111 were supplied as calcium; in another 95 out of 112 were supplied as magnesium; and in a third 47 out of 62 were supplied as sodium. Other treatments usually had 50 per cent. of the cations as sodium and 25 per cent. each as calcium and magnesium. Using data from 8 crops, some of them at 3 locations, and making comparisons at approximately equal osmotic concentrations yields were found to be slightly better when calcium was the predominant cation than when the ratio was 50 per cent. sodium, 25 per cent. calcium and 25 per cent. magnesium. The increase in yield due to a predominant calcium salt was particularly marked in the case of alfalfa.

Where magnesium was the predominant cation, yields were usually lower than when calcium or sodium was preponderant.

Where sodium constituted 76 per cent. of the cations present in terms of milliequivalents per liter, the yields were approximately the same as when the ratio of sodium to total cations was 50 per cent. Thus in sand cultures where the physical condition of the substrate is not a problem, sodium in the ratios used does not appear to be a particularly injurious cation for the plants grown.

Effect of climate in modifying the effect of salts on plant growth

It was believed that the effect of a certain salt concentration in reducing the relative yield would not be the same in two widely different climates. Thus if 2.4 atmospheres will reduce the yield of beans to 50 per cent. of normal at Riverside, should we expect the same yield reduction in a hotter climate where transpiration would be greater? This is an important question because if experimental data showed that climate did not modify the effect of salts on plant growth, it would be possible to carry on experiments at one location and expect similar results to be obtained under all climatic conditions.

In 1940 onions were grown at the three locations in a base nutrient solution, and in this base nutrient solution plus added chloride, sulphate-chloride mixture, and sulphate salts which had osmotic concentrations of 4.1, 4.3, and 4.5 atmospheres, respectively. Data on climate for the month of July at the three test locations are given in table II.

TABLE II

CLIMATIC DATA FOR INDIO, RIVERSIDE, AND TORREY PINES FOR JULY, 1940

LOCALITY	MEAN MAXIMUM TEMPERATURE	MEAN MINIMUM TEMPERATURE	AVE. EVAPORATION	AVE. RELATIVE HUMIDITY AT 8:00 A.M. & NOON		AVE. WIND	AVE. SUNSHINE
	° F.	*° F.*	*ml./100 sq. cm./day*	*%*	*%*	*mi./day*	*gm. cal./day*
Indio	112	71	1384	89	21	26	716
Riverside	97	56	1061	78	32	33	628
Torrey Pines	78	59	544	72	67	31	678

* Gram calories per sq. cm. of horizontal surface per day. The record for Torrey Pines was based on July 4 to July 21, 1940.

From data given in table II we note that sunshine at the three locations during July is nearly equal. At Torrey Pines, because of its location within 1 mile of the ocean, relative humidities are high and temperatures are equable. At Indio, on the other hand, temperatures are high, noon humidi-

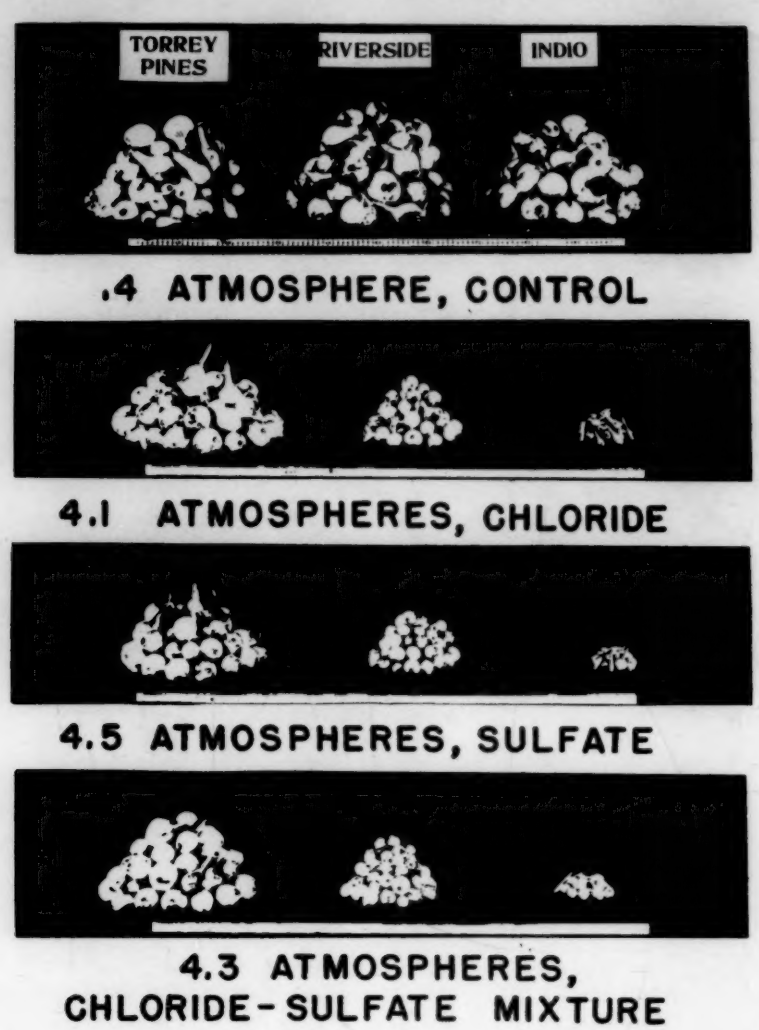

FIG. 6. Harvest yields of onions grown at three locations and at varying osmotic concentrations of substrate.

ties are low, and evaporation is high. Riverside has an intermediate climate with warm days and cool nights. Onions were grown during the period April 16 to July 26, 1940, and during the earlier part of this growth period the climatic conditions at the three stations were more similar than in July.

A picture showing onion yields is shown in figure 6.

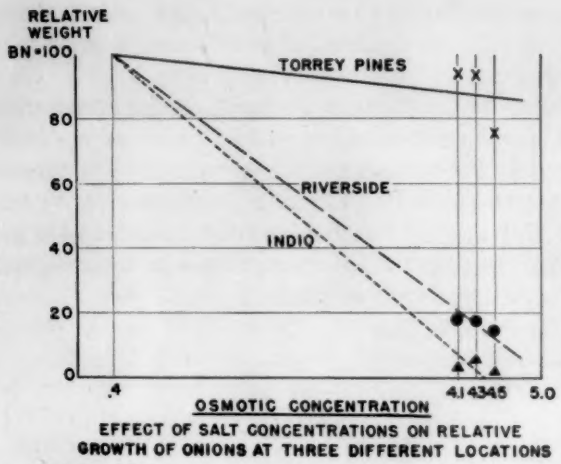

FIG. 7. Effect of salt concentration on relative growth of onions at three different locations.

One can compare the effect of salt, of climate, and of climate on salt effect at the three stations when the yields are placed on a common basis with

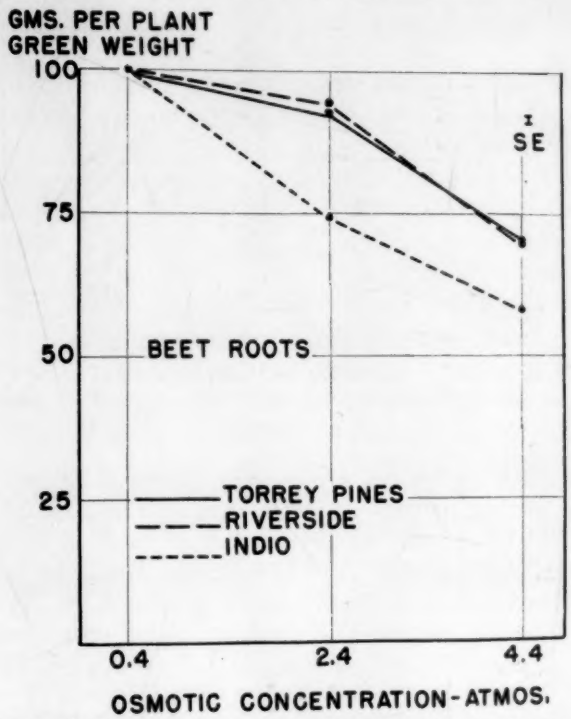

FIG. 8. Effect of salt concentrations on relative growth of roots of garden beets at three different locations. A measure of the accuracy is given by the length of the line S.E. (Standard error).

yield at each location in the base nutrient taken as 100. Actual yields in the base nutrient cultures at Indio, Riverside, and Torrey Pines were 5913, 6878, and 4577 grams of fresh bulbs respectively.

The data in figure 7 have been prepared on the relative basis and show that the yield of onions was only slightly reduced at the high salt concentrations at Torrey Pines. At Indio the reduction in relative growth at osmotic concentration of 4.1 to 4.5 atmospheres was very great, nearly 100 per cent. At Riverside the relative growth reductions were nearly as great as at Indio. This test involved three replicates and the regression lines are all significantly different from each other.

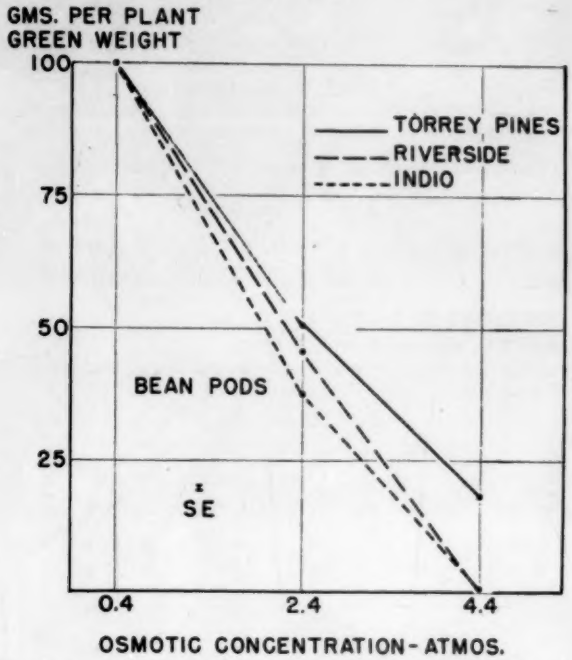

FIG. 9. Effect of salt concentrations on relative growth of beans at three different locations. A measure of the accuracy is given by the length of the line S.E. (Standard error).

When compared on this relative basis the curves in figure 7 show that in a cool climate salt concentrations in the range studied did not appreciably reduce the yield of onions. In the warmer climates as at Riverside and Indio the climate has affected the yield reduction caused by salt. In statistical terms there was a very significant interaction of climate on salt.

In 1941 additional data were obtained on beans and garden beets at the three climate locations. The data for chloride solutions are shown in figures 8 and 9.

The yields for garden beet roots shown in figure 8 indicate that on a relative basis the smallest yield reduction (base nutrient—4.4 atmospheres) occurred at Torrey Pines. On the same basis the greatest reduction in yield

with concentration was obtained at Indio. The difference in relative yield at 4.4 atmospheres at Torrey Pines and Indio was highly significant.

In the case of bean pods (fig. 9) the relative yield reduction at 4.4 atmospheres was almost the same at Riverside and Indio. The differences in relative yield between Torrey Pines and Indio were highly significant.

The data obtained in 1939 are subject to considerable variation but including only data and crops which are consistent the results for the three years can be tentatively summarized as follows:

1. A group of crops at a given salt concentration are depressed in relative yield more in warm than in cool climates. These include: in 1939, squash, and tomatoes; in 1940, onions, beans, sugar beets, alfalfa, and cotton; and in 1941, garden beets, carrots, and beans. Milo grown in 1940 grew best at Riverside and was least affected by salt at the Riverside climate.

2. Another group consists of crops for which no appreciable difference was found in the yield reduction by salt at various climates. This includes cowpeas and probably alfalfa. The data on these two crops were obtained in 1939. No alfalfa stand was obtained at Indio where daily maximum temperatures averaged 110° F.

Discussion

Best growth of a crop in nutrient solution takes place at a concentration of about 0.3 to 2.0 atmospheres, depending on the nature of the nutrient solution, volume of solution to crop, nature of crop, temperature, and other factors. It would appear that the optimum concentration should furnish an ample supply of nutrients, yet have such a low osmotic value that water absorption is not markedly reduced. In the present experiment the base nutrient solution yielded excellent crops with an osmotic concentration of 0.4 atmospheres. No tests were made with slightly greater or lower concentration. Eaton (5) has indicated that small concentrations of chloride salts up to 10 milliequivalents per liter tended to improve tomato growth. The results reported in this paper do not bear on this point, but in nearly every case yields at the 2.4 atmosphere concentration were lower than in the controls. An exception occurred in the case of cotton at Torrey Pines, where the higher salt concentrations made the cotton less vegetative and the yield of bolls was greater in the salt treatments than in the controls.

The relative reduction in yield with increasing salt concentration beyond an optimum is often linear when plotted on an osmotic basis. This agrees roughly with the work of Hayward and Long (6) with tomatoes. They found better growth, however, at 1.5 atmospheres than at 0.5 atmosphere, but their ratio of solution volume to growth produced was relatively low. In a base nutrient series where the osmotic concentration was achieved by using greater amounts of nutrient salts the growth was better than at comparable concentrations obtained by adding sodium chloride or sodium sulphate to a 0.5 atmosphere base nutrient solution.

EATON's (5) data can be replotted on an osmotic concentration basis. When this is done the relationship between osmotic concentration and yields of beans, milo, seed cotton, and tomato fruits is nearly linear. Sugar beets showed a decidedly greater tolerance to chloride than to sulphate salts at equal osmotic concentrations.

AHI and POWERS (1) grew salt grass, alfalfa, and strawberry clover in various dilutions of sea water. In order to plot their yield results against osmotic values the present authors determined osmotic concentrations of Pacific Ocean water at a number of dilutions. The results obtained did not agree with a linear relationship, growth being better than expected on this basis at the high concentrations.

Work at this laboratory has dealt primarily with chloride and sulphate salts. The results to date indicate that in general, the growth reduction of crops grown in saline solutions made up of chloride and sulphate salts of calcium, magnesium, and sodium is roughly proportional to the osmotic concentration or to the conductivity of the solution. Individual crops may fall out of line somewhat, but these deviations should be checked further. Specific ion effects undoubtedly exist, but for the salts studied they appear to be of a second order, compared to the matter of total salt concentration.

While the results obtained in sand cultures indicate that there is little difference in cation effects, these results are wholly tentative. It is believed that if the range of each cation is varied widely, marked plant responses will occur. Sodium has a very profound effect on soil structure and sodium saturated soil takes on physical and chemical characteristics which are not conducive to plant growth. For this reason results with sodium in sand cultures are not expected to carry over completely into soils. Just how osmotic values affect growth is not known, but undoubtedly water intake, turgidity, and root extension are involved, together with other salt effects on the root and within the plant.

It is of interest to note that the soil solution of productive soils may have an osmotic concentration exceeding 1 atmosphere at soil moisture contents within the wilting range. Soils marginal because of salinity may have osmotic concentrations as great as 10 atmospheres at these moisture contents (9). These soil solution concentrations are of the same order as the concentrations of the culture solutions used in these sand culture experiments.

The results obtained in this study show that most crops are injured by salt to a greater extent in warm than cool climates. The alfalfa data for 1939 gave no appreciable differences in the salt effect at Riverside and Torrey Pines. In 1940 when the alfalfa was seeded earlier in the season and good stands were obtained, decidedly poorer yields at the same salt treatments were obtained at Indio compared with Riverside and Torrey Pines. As in 1939, there was no appreciable difference in yields at Riverside and Torrey Pines in the comparable treatments.

The data of AHI and POWERS when plotted on a relative basis give

slightly, but probably not significantly, lower yields of alfalfa at warm temperatures in the presence of salt. Their cold temperature of 55° F. was about 10° less than the average temperature at Torrey Pines. These results would indicate that the temperature coefficient for salt injury in the case of alfalfa is low.

Because of the great differences in crop reaction to the combined effect of salt and temperature as shown in this paper it appears dangerous to extrapolate results from one set of climatic conditions to another.

Summary

1. Milo, cotton, alfalfa, sugar beets, barley, tomatoes, squash, cowpeas, onions, navy beans, garden beets, and carrots, were grown in large sand cultures in three diverse locations at various salt concentrations ranging from 0.4 to 4.5 atmospheres.

2. Total salt concentration expressed in atmospheres was a greater factor in determining the amount of growth reduction than effects caused by specific ions.

3. Growth reduction was in most cases linear with increasing osmotic concentration of substrate.

4. Conductance of the nutrient solution characterized the total salt effect probably as well as osmotic concentration.

5. Chloride and sulphate salts when compared on an equal osmotic basis, depressed growth to an equal extent with a number of crops. In the case of other crops, chloride salts were slightly more toxic than the sulphate at equal osmotic concentrations.

6. Within the ratios of cations used in the experiments, there was no great difference in the action of individual cations on plant response. Sodium did not appear to be an unduly toxic cation in sand cultures.

7. Crops do not behave alike in their reaction to the combined effect of salt and climate. Thus, some are reduced equally in relative yield at a given salt concentration irrespective of climate, while most crops at the same salt concentration are depressed in relative yield more in warm than in cool climates.

8. A number of crop species died in a culture solution having an osmotic concentration of 4.5 atmospheres.

Acknowledgment is made to other members of the Laboratory staff, particularly F. M. Eaton, J. W. Brown, W. E. Taggert, L. R. Weaver, and K. R. Goodwin, who assisted in this experiment.

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AMOUNT, DISTRIBUTION, AND SEASONAL TREND OF CERTAIN ORGANIC RESERVES IN THE ROOT SYSTEM OF FIELD BINDWEED, *CONVOLVULUS ARVENSIS* L.[1]

JOHN C. FRAZIER

(WITH SIX FIGURES)

Introduction

Field bindweed, *Convolvulus arvensis*, is recognized as one of the more serious of the noxious weeds in the central United States. Four of the five federal projects established to study control measures for this plant are located in this area (Hays, Kansas; York, Nebraska; Cherokee, Iowa; and Lamberton, Minnesota).

During the past two or three decades practical means of control have been studied at various places. Empirical methods, however, have not resulted in satisfactory control and there has been a trend to include in a general research program studies dealing specifically with the physiology of the plant, especially studies of its food reserves. The persistent nature of noxious perennial weeds is intimately related to the organic reserves stored in their roots. The study herein reported deals particularly with the organic reserves of the root system when undisturbed by cultivation.

A study of the nature of the root system (**10**) showed the advisability of dividing it into a number of portions for detailed studies of their organic reserves. Analytical determinations were made upon 7 of these portions found in the upper 3 feet of soil, to learn the amount and relative distribution of certain of the organic reserves during the growing season.

ENVIRONMENTAL FACTORS

The environmental factors of soil type, precipitation, and air temperatures under which this study was made are given in detail by FRAZIER (**10**).

Briefly, it may be stated that the soil was a deep Geary silt loam, no layer of which impeded root development. The rainfall the preceding year was 24.54 inches which was 74 per cent. of normal; while during the first 10 months of 1937 the precipitation was 20.88 inches, which was 69 per cent. of normal for this period. The air temperatures the preceding year showed great extremes. On 60 days, temperatures of 100° F. or higher were reached (the average number is 15). The summer of 1937 was less extreme with temperatures of 100° being attained on only 38 days. The growth of bindweed was definitely terminated on November 17 on which date the temperature fell to 12° F.

Materials and methods

PHYSICAL METHODS

The roots used in this study were obtained by a modification of the trench-

[1] Contribution no. 419 from the Department of Botany, Kansas Agricultural Experiment Station.

ing method developed by WEAVER (**25**). This involved the use of a hand pick and an ice pick to expose and free the underground parts after suitable trenches had been made by excavating operations.

Roots secured for analytical studies were taken twice a month—the first and fifteenth—from April 1, 1937, to October 1, 1937, with a fourteenth and final sampling on November 1, 1937. All roots were taken from the one area of well-established, undisturbed bindweed, which was subjected to normal root competition by a uniform, but sparse stand of other weeds. There was scarcely any competition for light as the bindweed shoots twined over the other weeds.

The material was divided into the 7 portions of the root system as collected and kept in moist cloths until it was taken to the laboratory. There it was washed free of soil particles, blotted dry with cloths, cut in pieces approximately one-fourth of an inch in length, and placed in individual wire trays.

The root material was killed in an electric oven at a temperature of 95° C. This ordinarily required from 20 to 30 minutes. It was then dried at 70° C. in an electric oven for at least 14 hours.

The material was ground in a food grinder and then reduced to 60-mesh size with a mortar and pestle. It was stored in sealed glass containers, then redried at 100° C. for 14 hours before samples were taken for analysis.

One hundred lineal feet of each of the 3 portions into which the vertical roots were divided and one hundred lineal feet of the lateral roots of comparable plants growing in the same area of land were excavated and weighed in late July, 1939.

CHEMICAL METHODS

The following fractions were determined in each of the 7 divisions into which the root system was divided: total nitrogen, protein nitrogen, nonprotein nitrogen, reducing sugars, total sugars, the starch-dextrin fraction, and the readily available carbohydrate fraction. All results are the averages of closely-agreeing duplicate analyses.

TOTAL NITROGEN.—The diphenylamine test (**13**) showed the material to be free of nitrates which agrees with the findings of BAKKE *et al.* (**3**) and the Kjeldahl-Gunning-Arnold method (**2**) as modified by MILLER (**14**) was used to determine the total nitrogen in 1-gram samples.

PROTEIN NITROGEN.—This fraction as determined is considered to include the unextracted nitrogen plus that coagulated by specific treatment and is the method that has been employed in this laboratory (**14**).

NONPROTEIN NITROGEN.—This was obtained by subtracting the percentage of the protein nitrogen from the percentage of total nitrogen.

The reducing sugars, total sugars, and the starch-dextrin fraction were determined from a 3-gram sample, using methods adapted from those employed in this laboratory (**14**). The sample was extracted with 100 ml. of 80 per cent. alcohol for 2 hours in a 70° C. bath, filtered, the filtrate used for sugar, and the residue for starch-dextrin determinations.

REDUCING AND TOTAL SUGARS.—The filtrate was freed of alcohol by evaporation and the resulting water solution was cleared by the use of neutral lead acetate, brought to volume, deleaded with sodium oxalate, the lead oxalate precipitate allowed to settle, and a 50-ml. aliquot pipetted off for reducing sugar determinations. One hundred milliliters of the remaining aqueous solution were pipetted into a 250-ml. volumetric flask, acidified with 2.5 per cent. hydrochloric acid and hydrolyzed for 5 minutes at 70° C., as described by LOOMIS and SHULL (**13**), nearly neutralized with 20 per cent. sodium hydroxide, made to volume and a 50-ml. aliquot taken for total sugar determinations.

STARCH-DEXTRIN FRACTION.—The residue of the original separation was transferred to a beaker by means of hot water, made to approximately 100 ml. volume and boiled for 2 minutes to gelatinize the starch. After cooling, 10 ml. of fresh filtered saliva and 1 ml. of toluene were added and the contents of the beaker stirred thoroughly.

The beaker, covered with a watch glass, was placed in an electric oven at 38–40° C. for 14 hours, checked as being starch-free with iodine, filtered into a volumetric flask, washed with hot water, cooled, cleared with neutral lead acetate, made to volume, deleaded with sodium oxalate, the lead oxalate allowed to settle, and a 200-ml. aliquot pipetted into an Erlenmeyer flask, acidified with 2.5 per cent. hydrochloric acid, refluxed for 3 hours, nearly neutralized with 40 per cent. sodium hydroxide, brought to volume in a 250-ml. volumetric flask, and a 50-ml. aliquot taken for the starch-dextrin fraction determination.

All carbohydrate fractions were determined gravimetrically by weighing the cuprous oxide directly. The conditions of MUNSON and WALKER (**15**) were followed for reduction. The d-glucose equivalent was obtained from the MUNSON and WALKER tables.

READILY AVAILABLE CARBOHYDRATE FRACTION.—This composite fraction was computed by adding the percentage of total sugars to the percentage of the starch-dextrin fraction.

All results were obtained in percentage and in grams per 100 lineal feet for each of the reserve food fractions in each of the 7 portions into which the plants were arbitrarily divided. The weight in grams per 100 lineal feet for any reserve food fraction was obtained by multiplying the weight of 100 lineal feet of the plant portion by the percentage of the reserve food fraction in that portion.

Experimental studies

NATURE OF THE PLANT

FRAZIER (**10**) has described in some detail the gross nature of the undisturbed bindweed plant grown from seed. In summary, it was found that the undisturbed root system may be divided into three natural portions: (1) primary vertical root, which is the original root of the plant developing from the radicle of the seed; (2) permanent lateral roots, those lateral roots

that develop more extensively and persist as permanent parts of the root system; and (3) secondary vertical roots which are the downward extensions from the permanent lateral roots after the latter have made 10 to 30 inches of horizontal growth.

In this study all permanent lateral roots of whatever order (**10**) are considered together, and likewise all secondary vertical roots are considered collectively, regardless of their order of development. The two types of vertical roots were each divided into the first-, second-, and third-foot divisions or portions. These 6 divisions and the lateral-root division constitute the 7 portions of the plant into which it was arbitrarily divided for the purpose of determining the distribution and trend of organic reserves during the growing season, April 1 through November 1. The so-termed "first foot" of the secondary vertical roots was considered to begin where the slender lateral root suddenly enlarged markedly. This "first foot" was the first 12 inches of root beyond that point. Ordinarily this portion was entirely contained in the first foot of the soil. All the material designated as lateral roots came from the first-foot soil level.

Organic reserves of the plant

Percentage basis.—These results are reported as the percentage of the reserve food fraction in the dry material of the root portion. It was not possible to report them as percentages of the total dry weight of the entire plant since under field conditions complete plants could not be secured in sufficient numbers.

It is recognized that percentages may not give a true picture of the changes that are occurring in the actual amounts of organic reserves in the plant. An increase in the actual amount of one fraction may bring about an "apparent" decrease in another fraction, when reported on a percentage basis, despite the fact that there has been no actual decrease in the latter fraction. Admittedly the changes from protein nitrogen to the nonprotein nitrogen fraction and vice versa, and from sugars to the starch-dextrin fraction and vice versa, will show apparent errors in these fractions. It is contended, however, that the total nitrogen and the total readily available carbohydrate fractions are fairly reliable indices, as such shifts would be occurring within these fractions and would not materially change their values.

Nitrogen fractions.—*The primary vertical roots.*—In figure 1[2] the data are given for the total nitrogen, protein nitrogen, and nonprotein nitrogen fractions, expressed in percentage on a dry weight basis, for the 3 portions of the primary vertical roots. There is a definite decrease from April 15 to June 15, the decrease being more marked in some portions. There is less protein nitrogen relative to nonprotein nitrogen in the second, and still less in the third foot portion. The total-nitrogen and protein-nitrogen fractions reached their seasonal low points on August 15 in the second and third foot levels.

[2] The data, other than tables I and II, on which the graphs of this paper are based, are on file at the Kansas Agricultural Experiment Station, available upon request.

The secondary vertical roots.—In figure 2 the data are given for the total nitrogen, protein nitrogen and nonprotein nitrogen, expressed in percentage on a dry-weight basis for the secondary vertical roots. There was the same early season decrease following April 15, as in the primary vertical roots. The low point in the total nitrogen and protein nitrogen fractions of these

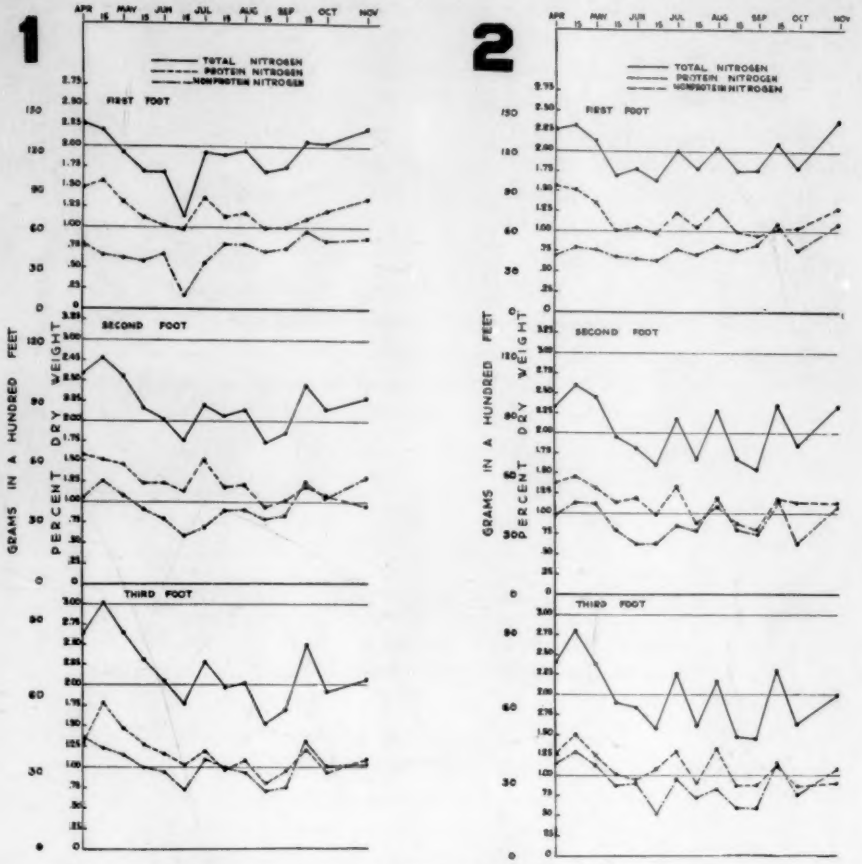

FIG. 1. The total nitrogen, protein nitrogen, and nonprotein nitrogen fractions in percentage on a dry weight basis and in grams per 100 lineal feet in the first, second, and third foot portions of the primary vertical root.

FIG. 2. The total nitrogen, protein nitrogen, and nonprotein nitrogen fractions in percentage on a dry weight basis and in grams per 100 lineal feet in the first, second, and third foot portions of the secondary vertical root.

roots centered, however, about September 1, except for the former in the surface foot. The trends after June 15 show more marked variations than those of the same fraction in the corresponding portion of the primary vertical root, but the general range of percentages does not differ markedly.

The lateral roots.—Figure 3 gives the data for the 3 nitrogen fractions in percentage of dry weight for the lateral roots. The early season decline is quite marked, especially in the total nitrogen fraction, due to the increase

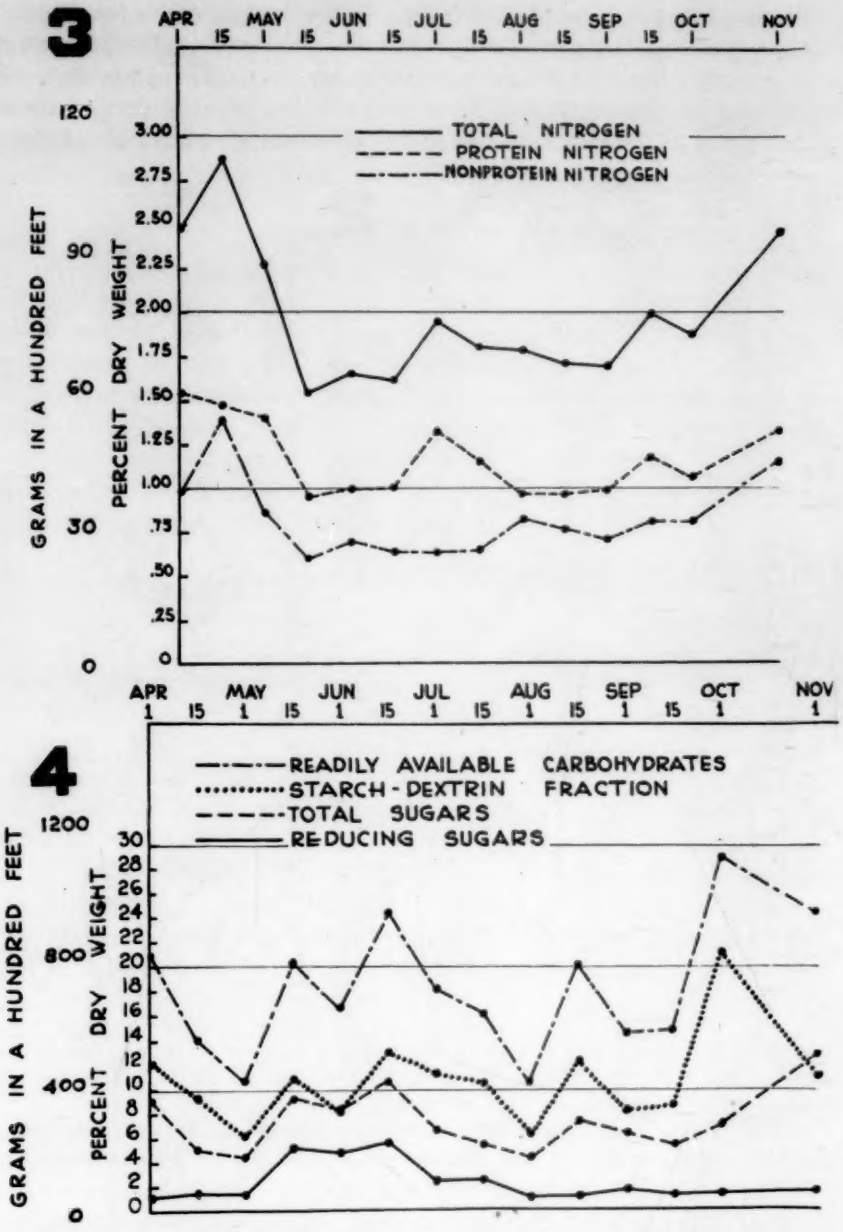

FIG. 3. The total nitrogen, protein nitrogen, and nonprotein nitrogen fractions in percentage on a dry weight basis and in grams per 100 lineal feet of the lateral root.

FIG. 4. The reducing sugars, total sugars, starch-dextrin fraction, and readily available carbohydrates in percentage on a dry weight basis and in grams per 100 lineal feet of the lateral root.

between April 1 and 15. Lows are found in the total-nitrogen and protein-nitrogen fractions in the months of May 15 to June 15 and August 1 to September 1. The general range of percentages of the 3 fractions is much the same as in the two types of vertical roots if the extremely low point of total proteins on June 15 in the primary vertical roots and the high point of this fraction on April 15 in the lateral roots are not allowed to bias the picture.

CARBOHYDRATE FRACTIONS.—*The primary vertical roots.*—Data are given in tables I and II for total sugars and the starch-dextrin fraction and in figure 5 for reducing sugars, total sugars, the starch-dextrin fraction, and the readily available carbohydrate fraction for the primary vertical roots.

The reducing sugars differed somewhat in their seasonal trend in the 3 portions. The early season low was from April 15 to May 15 in the first, from April 15 to May 1 in the second, and on April 1 in the third foot. There was a decrease in the first foot portion after July 15.

The total sugars showed an early season decrease in all portions. The seasonal low was reached by this fraction on May 15 in the first, on July 1 in the second, and on April 15 in the third foot portion. High levels were attained on June 15 and November 1 in the first, on June 1, September 15, and October 1 in the second, and on June 15 and August 15 in the third foot portion.

The starch-dextrin fraction reached a low point on May 1 in all three foot portions. This reserve fraction in the first foot portion was less than the total sugars on May 1 and June 15. The high points for the starch-dextrin fraction was on October 1 for the first, August 15 and October 1 for the second, and September 1 for the third foot portion. This fraction attained progressively higher percentages in the second and third foot portions.

The readily available carbohydrate fraction had the same general trend as the starch-dextrin fraction except between May 1 and July 1 in the first foot portion. It also attained progressively higher percentages in the second and third foot portions.

The secondary vertical roots.—Data are given in tables I and II for total sugars and the starch-dextrin fraction and in figure 6 for reducing sugars, total sugars, the starch-dextrin fraction, and the readily available carbohydrate fraction for the secondary vertical roots.

The reducing sugars differed in their trends from those in the corresponding portions of the primary vertical root. This fraction ran slightly higher in these roots than in comparable portions of the primary vertical root.

The total sugars reached low points on April 15, June 1, August 1, and October 1 in the first, on May 15 and October 1 in the second, and on May 15 in the third foot portion. High levels were attained on May 1, June 15, the month of August 15 to September 15, and November 1 in the first foot (the peak on November 1), on September 1 in the second, and on June 15 in the third foot levels. If the extremely low point reached in the first foot of the

TABLE I

TOTAL SUGARS IN PERCENTAGE AND IN GRAMS PER HUNDRED LINEAL FEET. MANHATTAN, KANSAS, 1937

DATE	PRIMARY VERTICAL ROOT						SECONDARY VERTICAL ROOT						LATERAL ROOT	
	1ST FOOT		2D FOOT		3D FOOT		1ST FOOT		2D FOOT		3D FOOT			
	%	gm.	%	gm.	%	gm.	%	gm.	%	gm.	%	gm.	%	gm.
April 1	6.04	374	8.86	360	8.86	286	8.08	500	8.72	354	9.92	320	9.12	346
April 15	4.75	294	6.42	261	5.17	167	5.75	356	7.83	318	8.46	273	5.00	190
May 1	5.33	330	5.37	218	7.17	232	8.12	503	6.45	262	11.25	363	4.29	163
May 15	1.96	121	5.83	237	8.12	262	7.38	457	6.01	244	5.21	168	9.23	350
June 1	6.62	410	9.54	387	9.92	320	5.33	330	8.21	333	12.66	409	8.23	312
June 15	8.42	521	7.25	294	11.50	371	8.58	531	8.21	333	13.20	426	10.96	415
July 1	3.31	205	4.29	174	10.00	323	6.20	384	8.71	354	9.54	308	6.98	265
July 15	6.96	431	8.42	342	9.79	316	6.79	420	10.76	437	11.33	366	5.96	226
August 1	7.08	438	7.38	300	10.08	326	5.04	312	8.33	338	8.80	284	4.04	153
August 15	6.79	420	9.13	371	12.63	408	8.88	550	9.13	371	9.08	293	7.78	295
Sept. 1	7.29	451	8.29	337	9.29	300	8.45	523	12.04	489	11.58	374	6.41	243
Sept. 15	7.21	446	9.46	384	7.54	244	8.88	550	9.13	371	9.59	310	5.71	216
Oct. 1	5.06	313	9.87	401	8.50	275	5.46	338	5.69	231	9.17	296	7.54	286
Nov. 1	9.54	591	9.17	372	8.13	263	9.58	593	9.91	402	10.50	339	13.21	501

TABLE II

STARCH-DEXTRIN FRACTION IN PERCENTAGE AND IN GRAMS PER HUNDRED LINEAL FEET. MANHATTAN, KANSAS, 1937

DATE	PRIMARY VERTICAL ROOT						SECONDARY VERTICAL ROOT						LATERAL ROOT	
	1ST FOOT		2D FOOT		3D FOOT		1ST FOOT		2D FOOT		3D FOOT			
	%	gm.	%	gm.	%	gm.	%	gm.	%	gm.	%	gm.	%	gm.
April 1	5.45	337	10.85	441	23.99	775	11.05	684	20.11	816	25.28	817	12.17	461
April 15	4.98	308	12.58	511	14.29	462	7.21	446	13.96	567	17.63	569	9.05	343
May 1	1.75	108	7.81	317	11.42	369	5.60	347	11.04	448	13.08	422	6.50	246
May 15	4.50	279	9.94	404	13.79	445	8.20	508	15.10	613	18.56	599	11.38	431
June 1	7.84	485	14.12	573	18.35	593	9.33	578	16.42	667	22.52	727	8.16	309
June 15	6.02	373	9.11	370	14.85	480	15.91	985	17.73	720	26.88	868	13.75	521
July 1	6.88	426	13.28	539	17.04	550	5.28	327	15.42	626	11.66	377	11.79	447
July 15	11.44	708	18.83	765	21.60	698	12.06	747	22.79	925	26.19	846	10.75	407
August 1	11.58	717	21.39	868	26.12	844	8.04	498	19.37	786	22.17	716	6.51	247
August 15	12.10	749	22.62	918	27.58	891	14.06	870	30.77	1249	38.27	1236	12.69	481
Sept. 1	7.92	490	19.83	805	31.95	1032	14.06	870	19.25	782	33.52	1083	8.05	305
Sept. 15	8.98	556	17.50	711	22.36	722	13.85	857	19.48	791	26.61	860	8.92	338
Oct. 1	15.15	938	22.50	914	27.56	890	21.42	1326	30.68	1246	34.93	1128	21.56	817
Nov. 1	8.92	552	20.23	821	25.96	839	12.37	766	24.55	997	31.10	1005	11.08	420

primary vertical root is overlooked, however, the range of percentages is similar in the two types of vertical roots.

The starch-dextrin fraction trends were downward in all portions until May 1. In the first foot portion, the starch-dextrin fraction was less than the total sugar fraction on May 1 and July 1. The general trends were similar to those of this fraction in corresponding portions of the primary vertical root, except that marked low points were reached in the first and third foot portions on July 1. The fraction, however, ran 4 to 6 per cent. higher

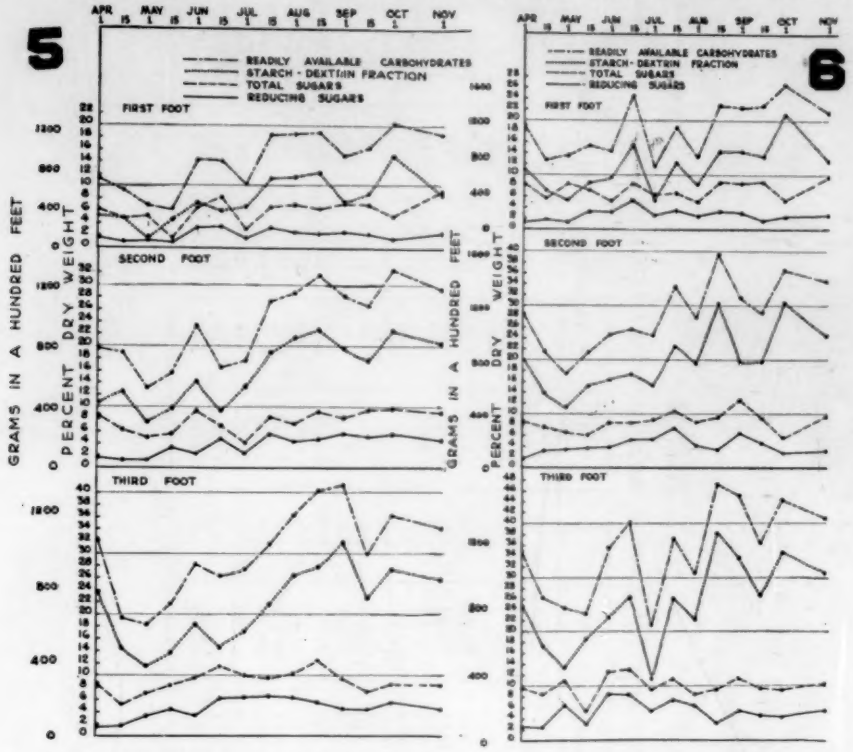

FIG. 5. The reducing sugars, total sugars, starch-dextrin fraction, and readily available carbohydrates in percentage on a dry weight basis and in grams per 100 lineal feet in the first, second, and third foot portions of the primary vertical root.

FIG. 6. The reducing sugars, total sugars, starch-dextrin fraction, and readily available carbohydrates in percentage on a dry weight basis and in grams per 100 lineal feet in the first, second, and third foot portions of the secondary vertical root.

in many instances than in the corresponding portion of the primary root.

The readily available carbohydrate fraction showed the same general trends as the starch-dextrin fraction in this root. This fraction ran 6 to 8 per cent. higher in many instances than corresponding portions of the primary vertical root.

The lateral roots.—Data are given in tables I and II for total sugars and the starch-dextrin fraction, and in figure 4 for reducing sugars, total

sugars, the starch-dextrin fraction, and the readily available carbohydrate fraction for the lateral roots.

The low point of reducing sugars occurred at the start of the study on April 1 and a second low interval existed from August 1 to August 15. The peak was attained on June 15. The general trend was similar as for this fraction in the first foot portion of the two types of vertical roots.

The total sugars reached low points on May 1 and on August 1 and attained high levels on June 15 and November 1. The general trend differed somewhat from those of this fraction in the first foot level of the vertical roots.

The starch-dextrin fraction dropped to low points on May 1 and on August 1. High points were reached on June 15 and August 15, but the peak was reached on October 1. On June 1 and November 1 this fraction was slightly less than the total sugar fraction.

The trend of the total readily available carbohydrate fraction practically paralleled that of the starch-dextrin fraction for this root. A high point was reached on June 15, with the seasonal peak being attained on October 1. The percentages of this fraction, as in the starch-dextrin fraction, more closely approximated those in the first foot of the secondary than in the first foot of the primary vertical roots.

BASIS OF ACTUAL AMOUNT.—The story of any reserve food fraction expressed on a percentage basis is confusing in that the percentage ordinarily increases with depth while the actual amount ordinarily decreases. The findings of BAKKE *et al.* (**3**) show these trends.

In order to give the results of this study in terms of the actual amounts of each of the individual reserve food fractions in each of the 7 portions into which the plant was arbitrarily divided, the weight of 100 lineal feet of each of the 3 portions into which the vertical roots were divided and 100 lineal feet of the lateral roots was obtained by excavating representative roots of comparable plants and weighing them. These weights were as follows:

Weight of 100 lineal feet

Vertical root	
1st foot portion	61.9 gm.
2d " "	40.6 "
3d " "	32.3 "
Lateral root	37.9 "

The weight of the individual root portion was then multiplied by the percentage of the reserve food fraction in that portion to give the actual weight of that reserve fraction in 100 feet of that portion. These data have been computed and they show that the picture of reserves from the standpoint of actual amounts is markedly different from that constructed on a percentage basis. A specific case involving the first and third foot portions of the primary vertical root for August 1 illustrates this point. On a per-

centage basis, the first foot had 18.66 and the third foot 36.20 per cent. of the total readily available carbohydrate fraction, while on the basis of the actual amount of this reserve fraction, the first foot had 1,155 grams as compared with 1,169 grams in the third foot. Hence, with almost twice the percentage of this reserve fraction, the third foot had but little more of the fraction.

Nitrogen fractions.—These computed data are given in graphic form. Figures 1, 2, and 3, inclusive, give total nitrogen, protein nitrogen, and nonprotein nitrogen fractions in the primary vertical roots, the secondary vertical roots, and the lateral roots in grams per 100 lineal feet.

Carbohydrate fractions.—The computed data for total sugars and the starch-dextrin fraction are given in both tabular (tables I and II) and graphic form (figs. 4, 5, and 6), and in graphic form only for reducing sugars and the readily available carbohydrate fraction in the lateral, the primary vertical, and the secondary vertical roots, respectively.

It is obvious because of the method of calculation that the trend of the data reporting the actual amount of a reserve food fraction in grams per 100 lineal feet of a root portion will be the same as that of those reporting the percentage of this reserve food fraction in the same root portion.

Statistical treatment of differences between comparable portions of vertical roots

The seasonal trend of the various carbohydrate and nitrogen fractions was essentially the same for the primary and secondary vertical roots, hence comparable foot portions of these roots were paired by sampling dates and the differences of these various fractions in the comparable portions treated statistically, using the t test, to ascertain if the differences were statistically significant and, if so, to what degree (**17**). The value of t was used to determine the probability (P) from the tables for P values given by Fisher and Yates (**9**). These values are given in table III.

These P values showed that the differences observed between comparable portions of the two types of vertical roots were statistically significant in many instances. The differences in the total nitrogen fraction in the second and third foot portions, the protein nitrogen in the second foot portion, and the nonprotein fraction in the first foot portion were at least significant. The reducing and total sugars were at least significantly different in the first foot portion, while the starch-dextrin and readily available carbohydrate portions were at least significantly greater in the secondary vertical roots in all three foot levels.

It would appear that the statistical significance of the differences warrants consideration. Under ordinary conditions of growth, however, the secondary vertical roots outnumber the primary ones from two to many times, which may mean that root samples taken from undisturbed bindweed are predominantly of the one type. Then, too, the effect of control procedures, such as cultivation, on these differences is not known.

TABLE III

THE t VALUE, PROBABILITY, AND DEGREES OF SIGNIFICANCE OF THE DIFFERENCES BETWEEN THE VARIOUS ORGANIC RESERVE FRACTIONS FOUND IN COMPARABLE FOOT PORTIONS OF THE TWO TYPES OF VERTICAL ROOTS PAIRED BY SAMPLING DATES

FOOT INTERVAL	AVERAGE OF RESERVE FRACTION FROM 14 DATES OF SAMPLING		t VALUE (13 df.)	P (PROBABILITY)	SIGNIFICANCE
	VERTICAL ROOT				
	PRIMARY	SECONDARY			
	NITROGEN FRACTIONS				
	Total nitrogen				
	%	%			
1	26.54	27.51	1.516	0.16	n.s.
2	30.75	28.62	3.656	0.005	†
3	30.43	27.80	4.893	<0.001	‡
	Protein nitrogen				
1	16.84	16.38	1.443	0.18	n.s.
2	17.29	15.91	3.091	<0.01	†
3	16.24	15.25	1.720	0.11	n.s.
	Nonprotein nitrogen				
1	9.70	11.13	2.484	0.03	*
2	13.46	12.71	1.288	0.22	n.s.
3	14.19	12.55	0.439	0.67	n.s.
	CARBOHYDRATE FRACTIONS				
	Reducing sugars				
1	31.68	37.67	19.810	<<0.001	‡
2	53.12	57.96	0.789	0.46	n.s.
3	63.83	74.29	1.399	0.19	n.s.
	Total sugars				
1	86.36	102.52	2.233	0.045	*
2	109.28	119.13	1.246	0.24	n.s.
3	126.70	140.29	1.605	0.14	n.s.
	STARCH-DEXTRIN FRACTION				
1	113.51	158.44	3.504	0.006	†
2	220.59	276.67	4.275	<0.001	‡
3	296.86	348.40	2.921	0.012	*
	READILY AVAILABLE CARBOHYDRATES				
1	199.87	260.96	3.858	0.004	†
2	329.87	395.80	5.751	<0.001	‡
3	423.56	488.69	10.689	<<0.001	‡

n.s. Nonsignificant.
* Significant at 5% level.
† Significant at 1% level.
‡ Significant at 0.1% level.
< Less than.

Discussion

Comparatively few specific data have been gathered on the food reserves of weeds. BARR (5) has summarized the investigations in this field to 1936.

The papers by ARNY (1) and WELTON, MORRIS and HARTZLER (26) are similar to the study reported in this paper in that they follow the nitrogen and carbohydrate fractions in the roots of perennial weeds, other than bindweed, through the growing season, but the work was not done on material obtained from definite foot intervals of the soil.

Until recently food reserve studies on bindweed have been of a general nature. BARR (5) published the first rather complete analytical study of bindweed reserves in 1936.

It is the intention here to point out certain pertinent findings and viewpoints which may be of value in relating the results reported in this paper to the problem of food reserves in perennial weeds, particularly field bindweed, rather than to compare these with the specific results of other workers.

NITROGEN FRACTIONS

ARNY (1) states that while nitrogen reserves are present in relatively small amounts in perennial weeds they are of great importance in the metabolism of the plant, particularly at the time of rapid tissue building in the wave of spring growth. Recent studies by TIMMONS (19, 20, 21, 22, 23), EVANS (8), BARR (6) and BAKKE *et al.* (3) indicate that the trend of total nitrogen reserves is independent of the trends shown by the various carbohydrate fractions; that the variation of the nitrogen fractions during the season is low; and that the trend of the total nitrogen fraction has little value as an index to the regenerative power of the plant. BAKKE *et al.* (3) state that their work indicates that nitrogen depletion, unlike carbohydrate depletion, was not directly affected by chlorate treatment.

CARBOHYDRATE FRACTIONS

LATSHAW and ZAHNLEY (12) made observations in 1927 on the starch reserves of bindweed, and CRAFTS and KENNEDY (7) stated in 1930 that the persistent vegetative activity of the plant is made possible by the storage of relatively large quantities of starch in the root system. BARR (5) published in 1936 the first detailed study of the carbohydrate reserves of the bindweed plant. This study, however, was on material obtained at one sampling date, *viz.*, August 5, 1935. Extensive work has been done on the trend of the carbohydrate fractions in undisturbed bindweed by the following workers on the federal bindweed project: BAKKE (4) at Hawarden and Cherokee, Iowa; EVANS (8) at York, Nebraska; SEELY (16) at Genesee, Idaho; STAHLER (18) at Lamberton, Minnesota; and TIMMONS (23, 24) at Hays, Kansas. A summarized report[3] of their work indicates that the computed fraction, the readily available carbohydrate fraction, has the same general trend at the 5 stations if the time of the start of spring growth and the subsequent stages of growth are taken into consideration. Consisting as it does of the total sugars and the starch-dextrin fraction, this computed

[3] Progress report of cooperative weed investigations. U. S. Dept. Agr., B. P. I., Washington, D. C. 1939. Mimeographed.

fraction was found by these investigators to be the most reliable index of the food reserves of the plant. BAKKE *et al.* (**3**) state that the reserves of the bindweed roots consist largely of sucrose and a dextrin-like compound or group of compounds. In the study reported herein these substances are included in the fraction designated as the readily available carbohydrates. BARR (**6**) gives the trends for the carbohydrate fraction in undisturbed bindweed for 3 years' investigations in Colorado. The trends for the starch-dextrin fraction and total sugar fraction ran quite similar to those reported in this paper. There was a difference in the reducing sugar trend, however. The total sugar percentages were much higher than those reported in this paper or by TIMMONS (**19, 20, 21, 22**).

Most of these analytical studies were on bindweed root material taken in the 0- to 12-inch or the 6- to 18-inch soil layer with certain exceptions such as: TIMMONS (**21, 22**) included five periods of sampling at what he termed "critical points in the seasonal curve for food reserves," taking samples from the 6- to 18-inch, 18- to 30-inch, and 30- to 48-inch soil layers; KIESSELBACH *et al.* (**11**) made "fodder" analyses of roots taken to a depth of 4 feet; BAKKE *et al.* (**3**) analyzed samples taken to a depth of 17 feet in 1936 and to a depth of 8 feet in 1937 as well as samples used in routine studies on the 0- to 12 and 12- to 36-inch soil layers. The study reported herein is believed to be the first in which detailed analyses were made on undisturbed bindweed roots from the first, second, and third foot soil levels throughout the growing season.

Summary

1. For the purpose of studying the distribution and seasonal trend of the various organic reserves in the roots during the growing season the root system was arbitrarily divided into the permanent lateral roots and the two types of vertical roots. The two types of vertical roots were each divided into the first, second, and third foot portions, which with the lateral roots gave a total of seven portions. Only roots of well-established, undisturbed plants were used, subject to but little competition from a sparse but uniform stand of other weeds.

2. The percentages of total nitrogen and protein nitrogen fractions in the seven portions of the bindweed root system at fourteen periods of sampling—April 1 to November 1, inclusive—were determined and reported.

3. A fraction obtained by subtracting the protein nitrogen fraction from the total nitrogen fraction was computed and reported. It is designated the nonprotein nitrogen fraction.

4. The general trend of the nitrogen fractions in practically all portions of the plant showed an early season rise to April 15, followed by a decline to a low point sometime during the interval, May 15 to June 15. Marked fluctuations occurred following this interval in many of the portions, succeeded by a second low interval, August 15 to September 1. In certain instances this was the low point of the season of the reserve fraction in that root portion. Additional fluctuations followed, which included a rise on

September 15 followed by a decline and then a rise on November 1. The general range of percentages was similar in the corresponding portions of the two types of vertical roots, and in the first foot portions of those roots and the lateral roots if the few extreme variations are not allowed to bias the picture.

5. The percentages of reducing sugars, total sugars, and starch-dextrin fraction in 7 portions of the bindweed root system at 14 periods of sampling —April 1 to November 1, inclusive—were determined and reported.

6. A composite fraction, consisting of total sugars and the starch-dextrin fraction, was computed and reported. It is designated the readily available carbohydrate fraction.

7. All of the carbohydrate fractions, except the reducing sugar fraction, reached low points in all of the root portions during the interval of April 15 to May 15. In many instances this was the low point of the season for that fraction in that portion.

8. The total sugars attained a seasonal maximum the first of November in the lateral roots and the first foot portion of the two types of vertical roots. In the other portions of the root system there was a tendency to attain a high point on or before July 15 and a maximum later in the season but prior to November 1.

9. The starch-dextrin fraction attained a maximum in the permanent lateral roots and in the first foot portion of the two types of vertical roots on October 1, and in the interval of August 15 to September 1 in the third foot portion of the vertical roots. The second foot portion of the vertical roots reached high levels on August 15 and October 1.

10. The readily available carbohydrate fraction followed the trend of the starch-dextrin fraction closely, attaining a maximum in the permanent lateral roots and the first foot portion of the two types of vertical roots on October 1, and in the second and third foot portions of the two types of vertical roots in the interval of August 15 to September 1, except in the second foot of the primary vertical root which attained seasonal high points on August 15 and October 1.

11. The total nitrogen fraction and the total readily available carbohydrate fraction are considered more reliable indices of the reserves of the plant than any of their component parts, the readily available carbohydrate fraction being the more reliable of the two.

12. The differences between comparable portions of the vertical roots were treated statistically. The t test was used to determine probability. Certain of the differences were statistically significant, some to a high degree. The effect of control procedures on these differences is not known.

13. All results were also computed and reported on a basis of the actual amount in grams per hundred lineal feet of each of the reserve food fractions in each of the root portions.

The writer acknowledges his indebtedness to Dr. C. A. SHULL and other members of the Department of Botany of the University of Chicago and to

Dr. E. C. MILLER, Kansas State College, for helpful suggestions during the course of this study.

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SOLUBLE CONSTITUENTS AND BUFFER PROPERTIES OF ORANGE JUICE[1]

E. T. Bartholomew and Walton B. Sinclair

(WITH THIRTEEN FIGURES)

Orange fruits undergo certain definite and progressive changes in chemical composition during growth and maturation. The rates at which these changes occur depend chiefly upon climatic and soil conditions, but they are also somewhat affected by the rootstock, as shown by Hodgson and Eggers (**15**), Sinclair and Bartholomew (**17**), and others. While studying the influence of rootstocks on the composition of citrus fruits, it seemed important to the authors to investigate the changes that occur in the soluble constituents of the juice during growth and maturity of the fruit, and, especially, to correlate the concentration of one soluble constituent with that of another for the purpose of determining their physiological and biochemical characteristics. The present studies were therefore undertaken.

It is well known that differences in the composition of citrus fruits are due not only to variety and similar factors, but also to the influence of the geographical location in which the fruits are grown, and to seasonal changes affecting the rate of the ripening processes. A study of the effects of these environmental factors on the composition and quality of the fruit has clearly demonstrated that, without actual investigation, it is not safe to conclude that the characteristics of the citrus fruits in one locality are the same as those of the fruits in another locality. For this reason it is of extreme importance to know the changes in soluble solids, total and reducing sugars, acids, and pH that occur during the growth and maturity of citrus fruits in the different citrus-growing areas of southern California. A knowledge of the interrelation of these particular constituents has served as a foundation upon which a more extended and specialized program of research on citrus fruits has been initiated.

The results reported in the first part of the present paper, which concerns the interrelation of juice constituents, are especially significant because they show the combined effects of different rootstocks, soils, and regional and annual climatic factors on the constituents of orange juice, over a period of several years. Data presented later in the paper show the effect of external factors and of some of the juice constituents on the buffer properties of the juice.

In southern California, navel oranges reach the stage of commercial maturity during a period extending from late November to January; Valencias reach this stage from late March to May, the exact time depending upon the locality in which the fruits are grown. The period of fruit sampling for

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the present studies extended for a considerable time both before and after the stage at which the fruit became commercially mature. Such a study shows the changes that occur in the fruit as it grows and matures.

Materials and methods

Fruit and juice samples

The major portion of the fruit samples for juice analysis were taken over a period of seven years (1936–1942) from one Valencia grove and one Washington Navel grove at Riverside (inland area) and from one Valencia grove near Tustin (coastal area). These groves had been set out for the rootstock studies previously mentioned. Each of the 14 plots of 10 trees each in the Valencia grove at Riverside represented a different rootstock. The 14 rootstocks were duplicated in the 14 similar plots in the Valencia grove at Tustin. Thirteen of these rootstocks and one other stock were represented in the 14 plots in the Washington Navel grove at Riverside. When the rootstock project was begun, great care was exercised in order to obtain uniform buds and stocks (**20**).

The remainder of the fruit samples were obtained over a period of four years (1939–1942) from a relatively large number of Valencia- and Washington-Navel-orange groves in coastal, inland, and intermediate areas of southern California. In these groves each sampling plot consisted of at least 25 trees.

In sampling, 6 average-sized fruits were picked from each of the 10 or more trees in each plot. The fruits on some trees were larger than those on others, but only fruits of average size on any given tree were chosen. In some of the tests for determining the accuracy of the method of sampling, the fruits were picked at random; in all other tests, however, 3 fruits were picked from the north side of the tree and 3 from the south side. Samples were selected in this manner because previous investigation (**5**) had shown that the soluble constituents of fruits from the south side of the tree are higher than those of fruits from the north side and that, as a rule, small fruits have a higher concentration of soluble solids than large fruits.

In all tests the fruits were halved and the juice was extracted from both halves by means of a hand reamer. Both halves of the fruits were used because it is now generally known (**5**) that there is a higher concentration of soluble constituents in the stylar half than in the stem half of the fruit. The various organic analyses of the juice were made after it had been thoroughly mixed and the portions for analysis had been strained and centrifuged.

The Navel fruits used for determining the buffer properties of orange juice were about three and one-half months beyond the initial stage of commercial maturity; the Valencia fruits were only about two to four weeks beyond this stage. The ratio of soluble solids to acids in the Navel-orange juice was 16.4 : 1; in the Valencia juice, it was 10.8–11.9 : 1.

Analysis of juice

Total soluble solids were determined with an Abbé refractometer, and the refractive indexes were converted to soluble solids by means of a sucrose table. Strictly speaking, therefore, the total soluble solids, as recorded in this paper, are in terms of the percentage concentration which would equal a sucrose solution having the same refractive index. Stevens and Baier (**19**) have shown that refractometer readings for orange juice are too low, mainly because of the citric-acid content of the juice. Since the corrections for orange juice, such as that used in the present experiments, are small (maximum, 0.31 per cent.), none were made. The general conclusions are the same as if the corrections had been made.

Total acidity, expressed as citric acid, was determined by titrating an aliquot portion of the juice with a standard solution of NaOH, with phenolphthalein as an indicator; all titrations were made in air. All pH values were determined with a Beckman glass electrode pH meter. Titration curves were experimentally determined on samples of juice free of pectin. The pectins were precipitated by pouring the juice (25 ml.) into 95 per cent. ethyl alcohol (100 ml.) and allowing the mixture to stand overnight at room temperature. The pectins were filtered from the solution and washed thoroughly with alcohol. The solution and washings were combined and distilled in vacuum at 55° C. to free the solution of alcohol. The course of the titration curve was determined on this alcohol- and pectin-free solution.

Titration curves were also determined on solutions of citric acid to which the ash from orange juice had been added. The ash was obtained by evaporating to dryness, on a steam bath, known portions of juice (25 ml.) which were subsequently charred and ashed below red heat. The ash was then transferred quantitatively to a beaker and sufficient citric-acid solution was added to make the volume and concentration equivalent to that of the original orange juice.

The sugar determinations were made by the Hagedorn and Jensen (**12, 13**) method as modified by Blish (**6, 7**). The strength of the reagents employed by Blish was satisfactory for determining the total reducing substances (reducing and total sugars as glucose) in unclarified orange juice when the values ranged from 3 to 10 mg. in 10 ml. of diluted citrus juice. The samples were diluted so that the values fell within this range. This method was used because comparative tests showed that it was more rapid than the best of the copper reduction methods and, at the same time, gave comparable results for the quantity of sugar in the sample. The standard HCl method was used to invert the sucrose. After adding the HCl, the solution was allowed to stand at room temperature (approximately 22° C.) for 24 hours before making the determination. Parallel tests showed that HCl was as reliable as invertase scales (Wallerstein's) for inverting the sucrose in orange juice. The sugar values presented in this paper are probably a little high because the samples of juice were not clarified before the sugar determinations were made. Samples of unclarified juice showed a

reducing-sugar content of approximately 6.50 per cent. and a total-sugar content of approximately 12.54 per cent., while the corresponding values for clarified samples of the same lot of juice were 6.36 per cent. and 12.26 per cent. The comparatively small increase in accuracy to be gained did not appear to justify the expenditure of the extra time and labor that would have been necessary to clarify each sample of juice. The glucose factor on the reagents was determined with a sample furnished by the National Bureau of Standards. The acid and reducing-sugar determinations were made within 3 hours, usually within 30 minutes, after the fruit was reamed.

Results

The soluble constituents in the juice

Since 80 to 85 per cent. of the oranges that reach the commercial channels of distribution are finally consumed as juice, it follows that the composition of the juice is more important, in relation to fruit quality, than the composition of the whole fruit. This fact has been recognized for many years by investigators in the citrus industry (**3**, **9**), and it has served as a basis for formulating the legal maturity test (ratio of soluble solids to acids) in use at the present time. In general, the ratio of soluble solids to acids in the fruit juice may be used as a test for maturity because the total soluble solids increase, and the acids decrease, during growth and maturation of the fruit.

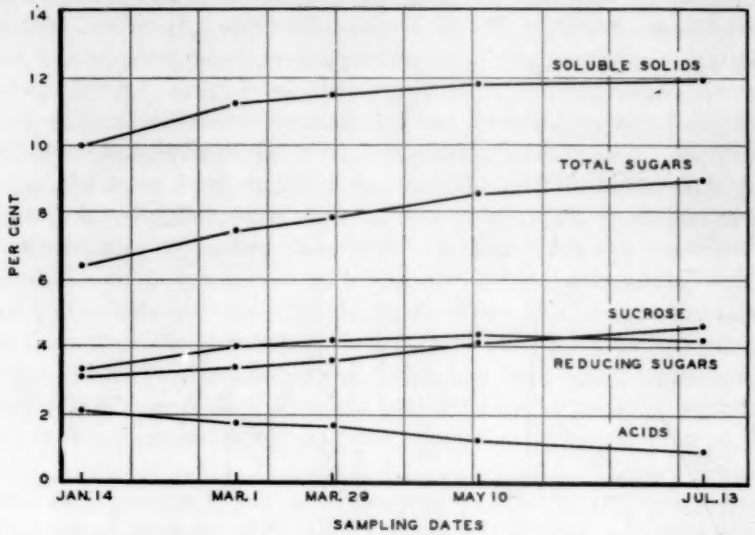

FIG. 1. Changes in percentages (fresh-weight basis) of soluble solids, total sugars, sucrose, reducing sugars, and acids of Valencia-orange juice with the advance of the season (January 14 to July 13). Each of the first four points on each curve represents the mean of 28 determinations made in 1939, on the date specified; the fifth point on each curve represents the mean of 72 determinations made on July 13 of three consecutive years, 1936, 1937, and 1938.

The data illustrated in figure 1 indicate the truth of the foregoing state-

ment in that the total soluble solids and the reducing and total sugars increased, while the titratable acidity decreased, at approximately the same rate, until May 10. After that date the soluble solids and total sugars increased more slowly and the sucrose and acids decreased. The reducing sugars alone continued to show the usual upward trend. These curves represent only the means of a large number of determinations. Later in this paper it is shown that there may be wide divergences from the means shown in figure 1. Although the curves were constructed from data based on determinations on Valencia-orange juice, Navel-orange juice was found to show the same trends. Each of the first four points on the curves (fig. 1) represents the mean value of 28 determinations obtained in 1939 from fruit from the Valencia plots located at Riverside and at Tustin. The fifth point on each curve represents the mean value of 72 determinations obtained from

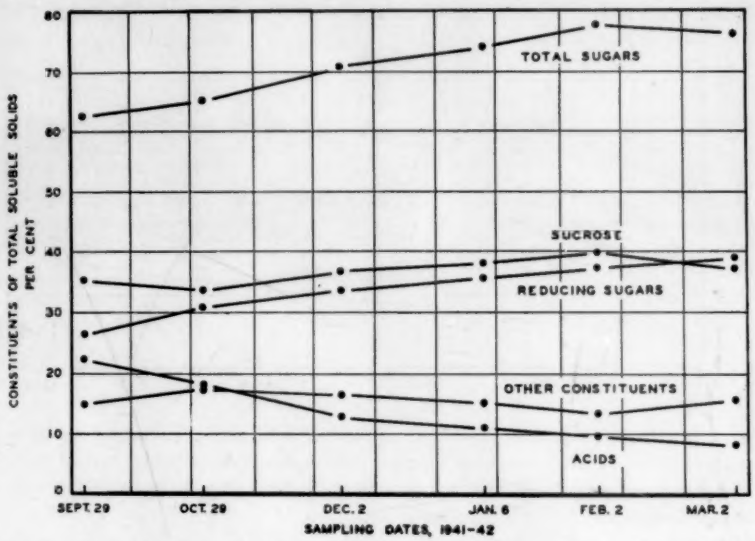

FIG. 2. Concentration of total sugars, sucrose, reducing sugars, acids, and other constituents in Navel-orange juice, expressed as percentages of total soluble solids (dry-weight basis), determined on samples picked at different times during the season. The maturity of the fruit ranged from approximately three months before the initial stage of commercial maturity to three months after.

the fruit from trees on the same Riverside and Tustin plots over a period of three consecutive years, 1936, 1937, and 1938.

The relative percentages of the different soluble constituents, based on total soluble solids, found in Navel-orange juice, at intervals from September 29, 1941, to March 2, 1942, are shown in figure 2. Total sugars increased from about 63 per cent. to a maximum of about 77 per cent. on February 2. The reducing sugars steadily increased over the entire period, from about 27 per cent. to about 38 per cent. Except for a drop on October 29, the sucrose increased from about 36 per cent. on September 29, to 40 per cent. on February 2. By March 2 it had dropped to about 37 per cent. Both of the

decreases in sucrose appear to be registered in the total-sugar values for these dates (October 29 and March 2). The acids decreased from about 23 per cent. to about 8 per cent. of the total soluble solids. The fraction of soluble solids designated as "other constituents," consisting of inorganic compounds, amino acids, ascorbic acid, a small amount of soluble pectins, etc., remained nearly uniform throughout the sampling period. The relative percentages of soluble constituents in Valencia-orange juice are similar to those in Navel-orange juice. The total sugars in the juice of fully mature Navel and Valencia oranges may comprise at least 75 to 80 per cent. of the total soluble constituents.

A comment should be made about the basis upon which the calculations have been made in figures 1 and 2. In the former, the constituents were calculated on the fresh-weight basis of the juice; in the latter they were cal-

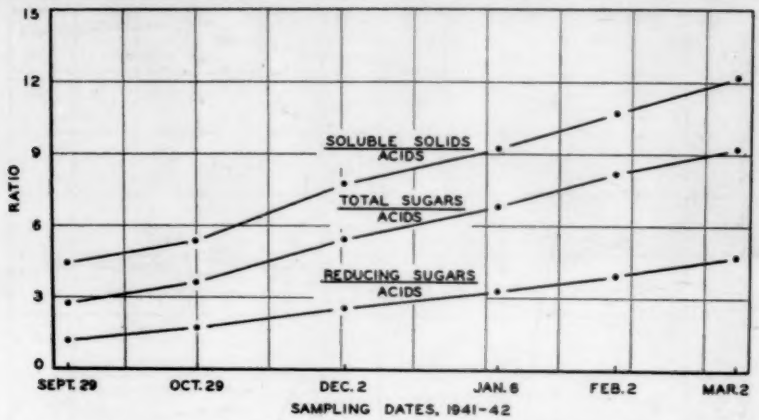

FIG. 3. Ratios of total soluble solids, of total sugars, and of reducing sugars to acids in Navel-orange juice, determined on samples picked at different times during the season. The maturity of the fruit ranged from approximately three months before the initial stage of commercial maturity to three months after.

culated on the dry-weight basis. It may be mentioned here that the figures for the percentage of total soluble solids in orange juice (fresh-weight basis) agree very closely with those for the dry weight of a sample of the same juice (**5**).

Since the total soluble solids, total sugars, and reducing sugars increased during the growing season, it is of importance to know the rate of change of these constituents with the change in concentration of acid. This has been graphically illustrated by plotting the ratios of these substances to total acidity against the time of sampling (fig. 3).

The ratio of soluble solids to acids serves, in part, as an index to maturity, for commercial purposes. It is difficult to attribute any great physiological significance to this ratio, however, as it is easily affected by very small changes in the acid concentration of the juice. Most of the soluble constituents in the juice are sugars, and this particular ratio is therefore often

referred to as the sugar-acid ratio. This usage is questionable, however, since the two ratios are very different, numerically and physiologically, chiefly because, as shown in figure 2, 10 to 15 per cent. of the soluble solids are not sugars and acids. Of interest is the fact that on September 29 the nonsugars included approximately 38 per cent. of the total soluble solids, while on March 2 they included only 24 per cent. (fig. 2). Practically all of this difference, however, was caused by the decrease in acids.

Although the ratio of total sugars to acids is not so high as that of soluble solids to acids, the rate of change during the season is about the same (fig. 3). The proportion of reducing sugars to acids shows a lower but similar relationship. These three ratios further demonstrate the inverse relation between the concentrations of soluble solids and sugars and the concentration of acids in the fruit, which signifies that, with increase in soluble solids and sugars, a corresponding decrease in concentration of acids occurs during the period of growth and maturation.

Relation of total soluble solids to total sugars, reducing sugars, and acids in the juice

Since a large number of determinations on the juice have been made in these studies, sufficient data are available to illustrate important and worth-

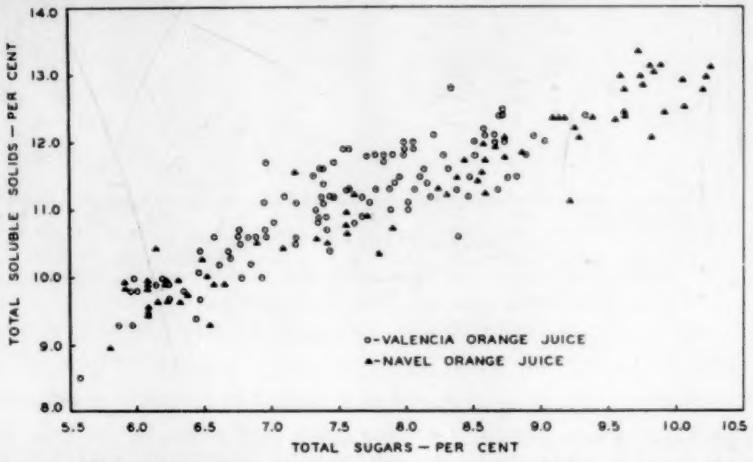

Fig. 4. Scatter diagram showing the relation of total soluble solids to total sugars in juice of Valencia and Navel oranges. Fruit maturity ranged from immature (green) to very mature (Valencias picked January 14 to July 13, 1939; Navels, September 29, 1941, to March 2, 1942).

while correlations of total soluble solids with certain other soluble substances in the juice.

In figure 4 the percentages of total soluble solids are plotted against percentages of total sugars of different samples of juice of Valencia and Navel oranges. The wide range shown (fig. 4) in concentration of total sugars, represents the amounts of sugar that occurred in fruits ranging from immature to fully mature. It is evident that within this range of fruit develop-

ment, the increase in total sugars parallels that of total soluble solids. These two variables should be closely correlated for the total sugars represent, on the average, from 63 to nearly 80 per cent. of the total soluble solids during the period over which this study was made (fig. 2). This is further evidence that soluble solids and total sugars, as already shown in figure 1, have a tendency to increase at about the same rate. There is sufficient scattering of the points (fig. 4), however, to show that the juice may contain 11.0 per cent. soluble solids, and that the total sugar content may range from 7 to 9 per cent.; or that, on the other hand, juice containing 8.5 per cent. total sugars may range from 10.5 to 12.5 per cent. soluble solids.

The concentration of reducing sugars in orange juice amounts to somewhat less than one-half of the total sugars, and the ratio remains fairly con-

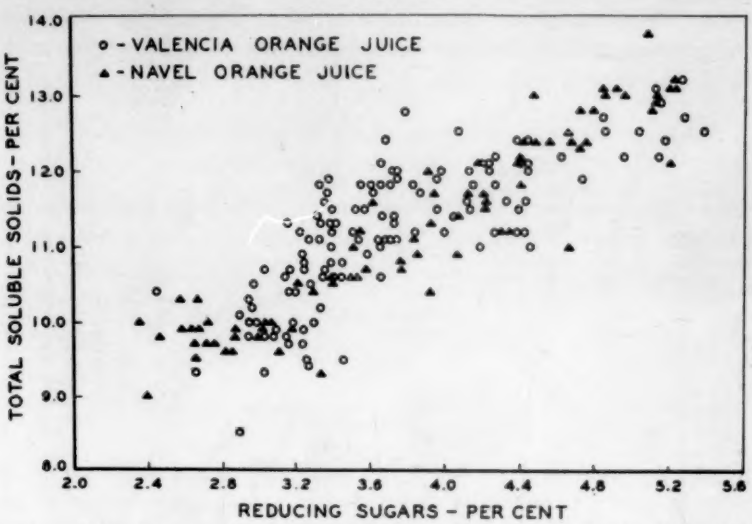

FIG. 5. Scatter diagram showing the relation of total soluble solids to reducing sugars in juice of Valencia and Navel oranges. Fruit maturity ranged from immature (green) to fully mature (Valencias picked January 14 to July 13, 1939; Navels, September 29, 1941, to March 2, 1942).

stant during the development and maturation of the fruit. It follows then that the relation between total soluble solids and reducing sugars (fig. 5) should be similar to that between total soluble solids and total sugars. Experimentally, however, the points for the values of reducing sugars showed a greater scattering on the diagram than those for total sugars. To illustrate (fig. 5), orange juice containing 11.0 per cent. total soluble solids may have reducing sugars ranging anywhere from approximately 3.2 to 4.5 per cent.; or, conversely, juice having reducing sugars of approximately 3.4 per cent. may contain total soluble solids of from 9.25 to 12.0 per cent. This may have been partly due to undetermined changes in the juice during the lapse of time between its extraction and analysis. Since in nearly all cases the reducing sugars were determined within 30 minutes after the juice was

extracted, it does not seem that this could have been an important factor. Part of the excessive scattering of the points for the reducing sugar values is probably due to the fact that this carbohydrate fraction is quickly and easily used in metabolic processes. From limited data obtained during the course of this experimental work, it appears that, under certain weather conditions, the concentration of reducing sugars is more variable than is that of nonreducing sugars.

It has already been noted that the sugars increase and the acids decrease in orange juice during growth and maturity of the fruit. The same trend prevails between total soluble solids and total acidity. Nevertheless, it is clear from the data in figure 6 that large changes can occur in total acidity

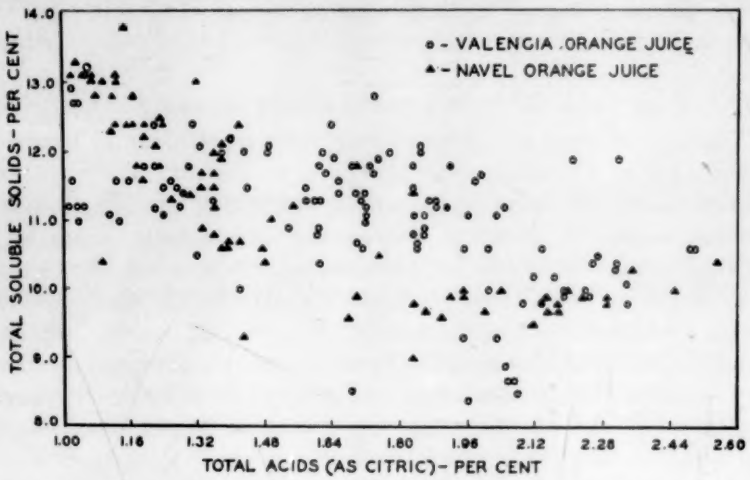

FIG. 6. Scatter diagram showing the relation of total soluble solids to total acids in juice of Valencia and Navel oranges. Fruit maturity ranged from immature (green) to fully mature (Valencias picked January 14 to July 13, 1939; Navels, September 29, 1941, to March 2, 1942).

with only a slight change in total soluble solids. The reverse is also true. This wide range in acid concentration (1 to 2.6 per cent.) shows that the juice samples were from fruits ranging from immature (green) to fully mature. These data explain why in many instances high concentration of soluble solids at maturity may not necessarily be correlated with a correspondingly low concentration of acids. Although it is generally true that the acids decrease and the soluble solids and sugars increase as the season advances, large variations in soluble solids and sugars of the juice frequently occur with only slight changes in total acids. For example, figure 6 shows that orange juice containing 11.0 per cent. total soluble solids may have a total acidity ranging anywhere from 1.00 to 2.60 per cent.; or, conversely, that juice having a total acidity of approximately 1.75 per cent. may contain anywhere from 8.5 to 13.0 per cent. total soluble solids. Results of this kind indicate the chances for error in using the ratio of soluble solids to

acids as the sole criterion of maturity of fruit and quality of orange juice. Slight changes in the percentage of acids result in large changes in the ratio without markedly affecting the quality of the juice. The practical aspects of these problems have long been recognized by the industry.

It may be well to point out here that the results shown in figures 1 and 7 appear to be at variance with the results obtained by CALDWELL (**8**). He reported that in oranges and grapefruit there is a progressive increase in both active and titratable acidity as growth advances toward maturity, but that as ripening begins, both active and titratable acidity start to decrease, and continue to decrease as ripening and maturity proceed. From a study of the two sets of data, it appears that CALDWELL terminated his experiments at about the same stage of fruit-ripening as that at which our experiments began; therefore, a direct comparison of the two sets of results cannot be made.

RELATION OF pH TO TITRATABLE ACIDITY OF ORANGE JUICE

pH AND TOTAL ACIDITY.—Juices of fruits and vegetables may have a comparatively low or a comparatively high total acidity and yet have approximately the same pH value, the condition depending upon the kinds and amounts of buffer salts present. To illustrate with an actual example, juice from Navel oranges grown on Rough-lemon rootstock, showed 0.80 per cent. total acids (in terms of citric acid), with a pH of 3.52, while juice from Navel oranges grown on Trifoliate orange showed 1.21 per cent. total acids, with a pH of 3.46. Although there was a 34 per cent. difference in the total acidity of these two juice samples, the pH values differed only slightly. These two samples of Navel oranges were picked on the same day (March 3, 1942) from two different blocks in the same grove.

During the course of this investigation, it was necessary to make a large number of determinations of pH and of total acids (titratable acidity), on juice of oranges from samples collected at various stages of maturity. These data, as represented in figure 7, show the relation between pH value and titratable acidity. It can be observed that these pH values were determined over a wide range of total-acid concentration (0.7 to 2.60 per cent.). Juice samples containing 2.60 to 0.70 per cent. total acids, represent fruits ranging from immature (green) to fully mature.

The data reported here (fig. 7) show that the pH value of orange juice bears a definite relation to the titratable acidity, if compared over a wide range of acid concentration. This relationship, however, is not so definite over shorter ranges of acid concentration, within which two samples of orange juice having rather large differences in total acidity may have the same pH value. For example, orange juice with a pH value of 3.20 may have a total acidity ranging from approximately 1.10 to 1.85 per cent.; or, conversely, juice with a total acidity of approximately 1.43 per cent. may yield pH readings ranging from 3.00 to 3.43. Under these conditions, the pH value does not indicate the amount of acid present, nor does a given acid value represent a definite pH value.

TITRATION CURVES OF ORANGE JUICE.—The relation of pH to titratable acidity (total acids) of orange juice can be further elucidated by following its resistance to changes in pH upon addition of increments of standard alkali to known amounts of orange juice. Orange juice, like all plant ex-

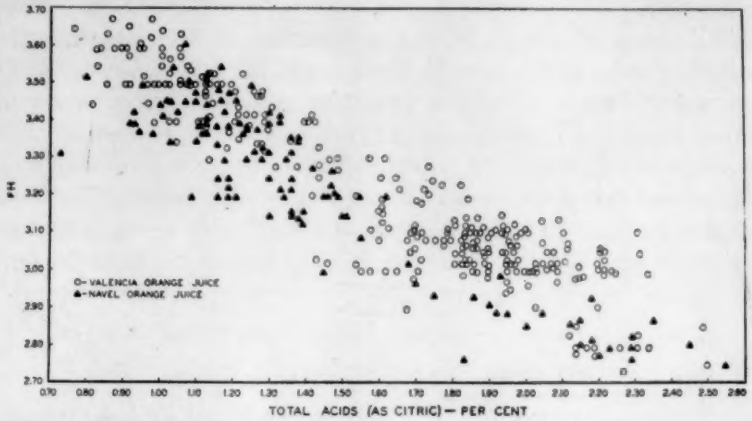

FIG. 7. Changes in pH and total acidity (titratable acidity) in juice of Valencia and Navel oranges as the season advances. As shown in figures 4, 5, and 6, a given value in one variable may represent many values in the other. Fruit maturity ranged from immature (green) to fully mature (Valencias picked January 14 to September 5, 1939; Navels, September 29, 1941, to March 25, 1942).

tracts, is, to a varying degree, capable of resisting changes in pH upon the addition of strong acids or bases. It was therefore decided to determine experimentally what substances were responsible for the buffer effect of the juice. The results of these experiments are given in the following paragraphs.

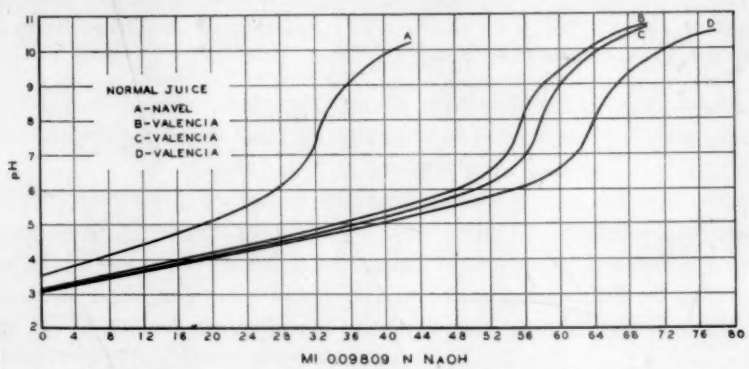

FIG. 8. Titration curves of various samples of Navel- and Valencia-orange juice containing different amounts of total acids. The ratio of soluble solids to acids was 16.4: 1 for Navel juice, and 10.8–11.9: 1 for Valencia juice.

An inspection of the buffer curves of different samples of normal orange juice (fig. 8) shows that the four samples did not contain the same concentration of acid. The endpoints calculated from curves A, B, C, and D occurred at pH 7.82, 7.81, 7.85, 7.83, respectively, but the differences in milli-

liters of 0.09809 N NaOH necessary to arrive at the pH of the endpoint show the differences in the amount of acid in the juices. If these juices, which were from fruits from different trees in the same grove, had possessed the same concentration of acid, all four of the curves would have been very close together.

In the course of this study, it was important to determine the effect of the soluble pectins in the juice on the shape of the buffer curve. The titration curves of orange juice from which the pectins had been removed, and of normal orange juice, are shown in figure 9, A and B, respectively. These two curves are very close. The slight difference between them may be partly due to the fact that a very small amount of a volatile acid was distilled with the alcohol during vacuum distillation, and partly due to the lack of pectin, which reacts with NaOH beyond pH 7. The course of the buffer curve of

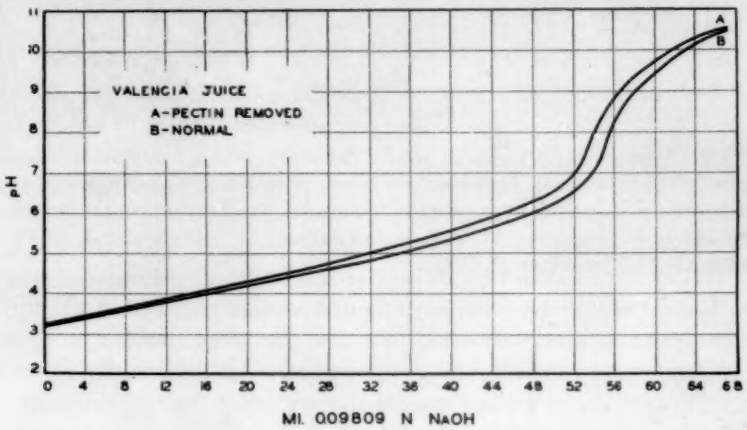

FIG. 9. The effect of pectins on the buffer action of Valencia-orange juice: A, pectins removed; B, normal. Both samples were from the same lot of juice, in which the ratio of soluble solids to acids was 10.8: 1.

the pectin-free juice definitely shows, therefore, that these pectin-free samples required less alkali for neutralization and that they apparently contained slightly less acid than those of normal juice.

Other titration curves were determined on normal juice only (fig. 10, C); on aqueous solutions of citric acid of a concentration equivalent to the amount calculated from the neutralization point on the curve of the normal juice (fig. 10, B); and, finally, on citric-acid solutions, with total acidity equivalent to that of the juice, plus the ash elements from a 25-ml. portion of the normal juice (fig. 10, A). A comparison of the buffer curve of citric acid (fig. 10, B) with that of normal Valencia-orange juice (fig. 10, C) shows that citric-acid solution has less buffering effect than orange juice. It is interesting to note, also, that the pH of the citric-acid solution is much lower than that of the juice. On the addition of ash elements to the citric-acid solution (fig. 10, A), the pH immediately increased from 2.07 to 3.10, the latter value being nearly the same as that of the original juice. This was

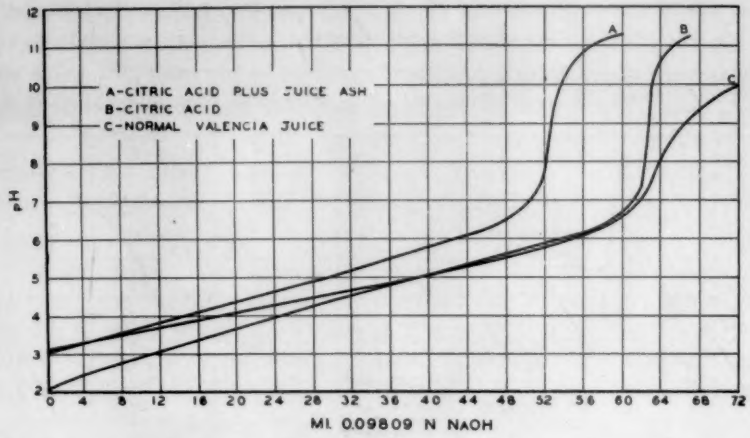

FIG. 10. Comparison of the buffer properties of (A) pure citric acid plus the ash elements from a 25-ml. portion of Valencia-orange juice, (B) pure citric acid, and (C) normal Valencia-orange juice. The ratio of soluble solids to acids for the normal Valencia juice was 11.2: 1.

caused by the reaction of the citric acid with the base constituents of the ash.

The titration curves of normal Valencia juice and of citric acid containing sodium citrate are shown, for comparison, in figure 11. The concentration of the citric-acid solution (25 ml.) used was equal to the total acidity calculated from the endpoint of the orange-juice curve. As usual, the

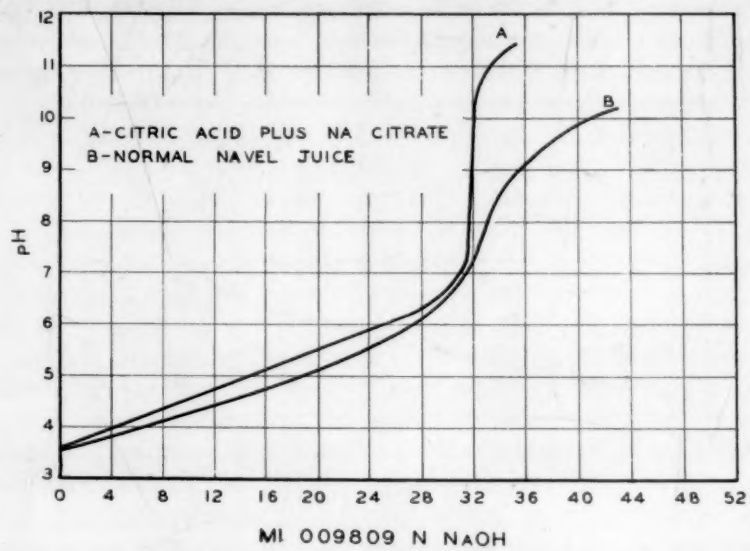

FIG. 11. A comparison of the buffer properties of citric-acid solution and Navel-orange juice: A, pure citric acid (of the same concentration as that of normal Navel-orange juice) to which had been added enough sodium citrate to bring the acid solution to the same initial pH value as that of the orange juice; B, normal Navel-orange juice. The ratio of soluble solids to acids for the normal Navel juice was 16.4: 1.

initial pH of the acid solution (fig. 11, A) was lower than that of the juice (fig. 11, B). Before the titration was started, a few drops of sodium citrate solution were added to bring the acid solution to the same pH value as that of the juice. The course of the titration curve of the acid solution differs

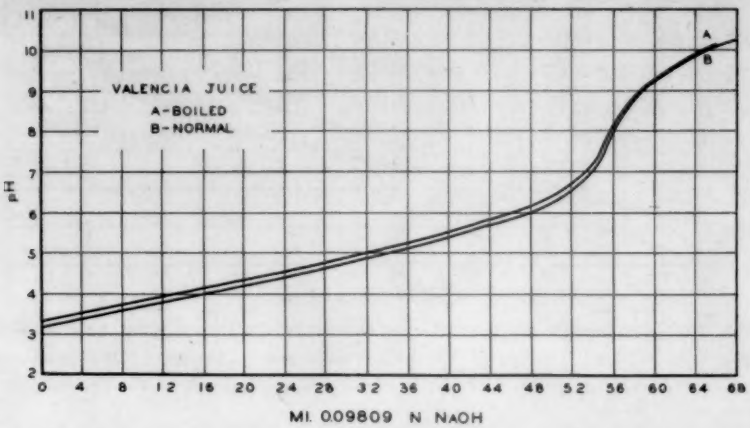

FIG. 12. The effect of heating on the buffer capacity of Valencia-orange juice: A, juice boiled for 45 minutes; B, not boiled. Both samples were from the same lot of juice, in which the ratio of soluble solids to acids was 11.9: 1.

only slightly from that of the normal juice up to pH 7; but, for some undetermined reason, beyond this point they show considerable divergence.

The effect of heat on the buffer system of orange juice (fig. 12) is shown by the titration curves for normal Valencia juice (fig. 12, B) and for a portion of the same juice boiled for 45 minutes (fig. 12, A). The boiling of

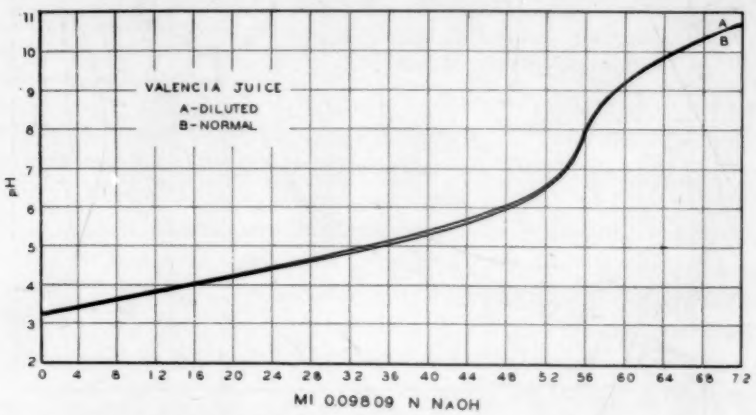

FIG. 13. The effect of dilution on the buffer properties of Valencia-orange juice: A, diluted (1 part juice to 4 parts distilled water): B, undiluted. Both samples were from the same lot of juice, in which the ratio of soluble solids to acids was 10.8: 1.

the juice apparently drove off a slight amount of a volatile organic acid or CO_2 to give a slight increase in pH and a slight lowering of the titratable acidity. These small differences were not sufficiently large to cause significant changes in the values or to alter the shapes of the curves.

The effect of heat on the small amount of soluble pectins in the juice was not reflected in these results. The boiling of pectin solution has the tendency to raise the free acidity of the solution slightly and to result in a certain amount of demethylation. In the systems reported here, factors other than pectin were in effect, for there occurred a slight loss, rather than an increase, in acidity.

The high degree of resistance to changes in pH is shown by the fact that orange juice can be greatly diluted without changing the pH or the shape of the titration curve (fig. 13). Although the limits of dilution were not reached in these experiments, titration curves are shown for normal orange juice (fig. 13, B) and for juice diluted in a ratio of 1:4 (fig. 13, A). This partially explains why the concentration of soluble constituents of orange juice can undergo relatively large changes during the growth and maturity of the fruit, with relatively small fluctuations in pH.

Discussion

In order to determine whether 6 fruits from each of the 10 trees in each plot were enough to give reliable results, two tests were made. In the first test, samples of 6, 12, and 18 fruits were picked (half of each sample from the north side of the tree and half from the south side) from each of the 10 trees in one of the plots in the Valencia grove at Riverside. The 6-fruit samples were combined to form sample 1 (60 fruits); the other samples were similarly combined to form sample 2 (120 fruits) and sample 3 (180 fruits). All fruits in each of the three samples were juiced. The percentages of total soluble solids in the juice from samples 1, 2, and 3, were 13.12, 13.12, and 13.07, respectively, a range of 0.05 per cent. In the second test, samples of 6 fruits and 40 fruits (samples 4 and 5, respectively) were picked from each of 10 trees in another plot in a different part of the same grove. All 60 fruits in sample 4 were juiced, but the juice for sample 5 was obtained from only 100 of the 400 fruits in the sample. The 100 fruits were taken at random, by a blindfolded person, from the 400 fruits after they had been thoroughly mixed. The percentages of total soluble solids in the juice from samples 4 and 5 were 12.97 and 12.84, respectively, a difference of 0.13 per cent. The differences in the values shown in these two experiments (0.05 and 0.13 per cent.) were found to be statistically insignificant.

The results of the two preceding tests were confirmed by still another test, in which 24 average-sized mature fruits were selected (12 from the north side of the tree and 12 from the south side) from each of 10 Valencia-orange trees in a different grove, from which no fruits had been picked during the current season. The 24 fruits from tree 1 were segregated at random into four groups (*a*, *b*, *c*, and *d*) of 6 fruits each. The 24 fruits from each of the other 9 trees were similarly segregated. By this method, each of the combined groups (*a*, *b*, *c*, and *d*) contained 60 fruits, 6 fruits from each of the 10 trees, as in our regular method of sampling. The total soluble solids for each group of 6 fruits from each tree were determined

separately, however; a total of 40 determinations. The mean percentages and standard errors of means for the total soluble solids were then calculated for each of the four groups of 10 determinations and were found to be as follows: group *a*, 13.36 ± 0.166; group *b*, 13.39 ± 0.176; group *c*, 13.26 ± 0.173; and group *d*, 13.29 ± 0.146. The difference between the means of the highest and lowest values is only 0.13 per cent., which is statistically insignificant, as shown by the *t* test at the 5 per cent. level and 18 degrees of freedom (**18**).

The results obtained in the plot method of sampling used in these tests, should not be given the same interpretation as those obtained by DENNY (**10**) and by APPLEMAN and RICHARDS (**1**), who made single-tree determinations. The results shown in the two preceding paragraphs indicate that the general method used in these experiments, in which 6 fruits were selected from each tree, were enough to give a representative sample from each plot of 10 or more trees.

Further comments should be made on the data of figures 1 to 7. Figures 1 and 2 illustrate the types of curves usually constructed to show changes that occur in the composition of fruits during growth and maturity. As only mean values are reported, the information revealed is general and satisfactory for showing the comparative seasonal trends in fruit composition. The curves fail, however, to yield information about variability between different constituents, and about the ranges in concentrations of various soluble solids in the juice during this same period of growth. Some of these relationships are clearly shown in figures 4 to 7, inclusive. It can be observed that, as in most biological data, the total sugars, reducing sugars, and acids in the juice do not bear a linear relation to the total soluble solids. Attention has already been drawn to the variations in amounts of these constituents with the advance of the season. The scattering of the points on the diagrams (figs. 4, 5, and 6) demonstrates the differences in the relative proportions of soluble constituents in the juice. Although the trend of the curves supplies enough information for the formulating of certain generalizations, the dispersion of the values is so great that this should be considered before making such generalizations. The condition is well illustrated by the changes in total soluble solids, with corresponding changes in total acidity, shown in figure 6. In this case, it can be seen that, with a given concentration of either constituent, there results a wide fluctuation in ratios.

The data for figure 4 came from the rootstock plots. In order to determine whether such conditions apply to southern California in general, a large number of similar determinations were made on fruit from groves chosen at random in coastal, inland, and intermediate areas. A scatter diagram of these results has not been included because of the already large number of figures. The data, however, portrayed a type of scattering that was identical with that shown in figure 4. The same comments apply to figures 5 and 6.

Although there is a more or less marked degree of scattering of the points

in figures 4 to 6, the degree of closeness with which any two of the soluble constituents may be related is more definitely expressed by their correlation coefficients, which have been calculated and are shown in table I.

The highest correlation coefficients obtained were those between total soluble solids and total sugars in the juice of fruit of Washington-Navel- and Valencia-orange varieties of the inland area (table I). The correlation of these constituents in juice of Valencia-orange fruits from the coastal area was lower in value. As shown in figure 6, the increase in percentage of total soluble solids and the decrease in percentage of acids do not occur at a uniform rate during the season. This is further emphasized by the significant but low negative correlations between these two variables, in samples

TABLE I

CORRELATION COEFFICIENTS SHOWING THE DEGREE OF CORRELATION OF VARIOUS SOLUBLE CONSTITUENTS IN ORANGE JUICE

FACTORS CORRELATED	VARIETY OF ORANGE	GROVE LOCATION, SOUTHERN CALIFORNIA	NUMBER OF SAMPLES n	CORRELATION COEFFICIENT* r
Total soluble solids and total sugars	Washington Navel	Inland area	79	+0.9700
	Valencia	Inland area	72	+0.9219
	Valencia	Coastal area	56	+0.8024
Total soluble solids and total acids	Valencia	Inland area	56	−0.5390
	Valencia	Coastal area	56	−0.5555
	Valencia	Random†	21	−0.4386
Total soluble solids and reducing sugars	Valencia	Inland area	56	+0.8159
	Valencia	Coastal area	56	+0.7096
Total sugars and reducing sugars	Valencia	Inland area	56	+0.9028
	Valencia	Coastal area	56	+0.8344

* These correlation coefficients are significant, as shown by the t test (18).
† Commercial groves in coastal, inland, and intermediate areas.

from different locations. Very high correlations existed between total sugars and reducing sugars during the period in which these samples were taken, the fruit samples of the Valencia variety from the coastal area yielding a lower correlation than those from the inland area. Data are also reported on samples from groves chosen at random in different citrus-growing areas of southern California. These data are included (table I) for purposes of comparison, in order to demonstrate that these experimental results are applicable in principle to fruit grown for commercial purposes.

The correlation coefficient for the relation of pH to total acids (fig. 7) was not calculated, but the degree of scattering of the points is such that a significant negative correlation is definitely indicated.

The relation of pH to the titratable acidity of orange juice is important in that it is, in general, related to fruit maturity; it is important, also, in that large changes in acid concentrations can occur with only very slight

changes in pH (fig. 7). This characteristic of orange juice is exhibited to a greater or lesser degree by most acid fruits. A quantitative measure of this characteristic may be obtained by observing the change in pH value of the juice upon the addition of increments of standard NaOH, as reported elsewhere in this paper. An inspection of figure 7 shows that at a given pH value, the juice may have various concentrations of total acids. If a range of acid concentration is chosen, for example, a range of 0.70 to 1.00 per cent., such as often occurs in fruit that is commercially mature, it is difficult to detect a direct relation between pH and total acidity. Over the total range of acid (0.70 to 2.60 per cent.), shown in figure 7, the pH tends to decrease with an increase in concentration of acid. In this particular instance, the relation of pH to titratable acidity depends upon the acid range. Other investigators with other fruits have found varying degrees of correlation between these two variables. In studies with 33 varieties of mature Minnesota apples, BARNES (**4**) obtained a high correlation coefficient ($r = 0.9265$) between titratable acidity and hydrogen-ion concentration of the juice; with 11 varieties of mature grapes, a high correlation ($r = 0.9213$) was also obtained; with 11 varieties of plums at different stages of maturation, the correlation ($r = 0.6198$) was much lower. ASKEW (**2**), on the other hand, did not find a definite relation between pH and titratable acidity (total acids) of mature apple juice.

The following information is of interest in that it indicates the differences in the proportionate amounts of reducing sugars and sucrose that may exist in orange juice as the season advances. As shown in figures 1 and 2, the reducing sugars comprised *less* than half the total sugars up to the time of making the last determinations—July 13 for Valencias (fig. 1) and March 2 for Navels (fig. 2). On these dates the reducing sugars amounted to *more* than half the total sugars. That this reversal was not a matter of chance is indicated by the fact that, when determinations were made on the juice of Valencia oranges from the 14 different rootstocks in the inland area (Riverside), on September 14 and 15, 1942 (approximately five and one-half months after the fruit had become commercially mature), the reducing sugars, in every case, amounted to more than half the total sugars. The average for the 14 determinations was 5.76 per cent. reducing sugars and 4.75 per cent. sucrose (by difference). The largest and smallest differences were 2.20 and 0.48 per cent., respectively. The results of determinations made on the juice of Valencias on the same kind of rootstocks in the coastal area (Tustin), on September 22, 1942 (approximately four and two-thirds months after the fruit had become commercially mature), were similar, but the differences were not so great. The averages were 5.00 per cent. for reducing sugars and 4.55 per cent. for sucrose. In two of the determinations, however, the percentages for reducing sugars (4.65 and 4.72) were about the same as those for sucrose (4.66 and 4.88), or were a little less. These results, which show a tendency toward increase in reducing sugars and decrease in sucrose in Valencia-orange juice as the season advances, are in line with the results

obtained by HILGEMAN and SMITH (14) on grapefruit juice, although the ratios of reducing sugars to sucrose in the grapefruit juice were higher than those in the Valencia juice.

The titration curve of orange juice shows that the buffer system is governed largely by organic acids and by salts of the inorganic constituents in the juice. Hydrolyzed ascorbic acid would furnish acids that would have a buffering effect, but the total acidity in orange juice is due mostly to citric acid. The small amount of soluble pectins has little if any effect on the buffer capacity of the juice, though pectin reacts with NaOH beyond pH 7 to form sodium pectate. Even in weak acid solution, pectins will form a loose combination with cations from neutral salts of calcium, sodium, and potassium. In alkaline solution, considerable time is required for pectin to react completely with NaOH to form sodium pectate. Soluble nitrogen compounds, in the form of amino acids and amides, exists in orange juice in such small amounts that they are of only slight importance in the buffer system. The dissociation constant of sugars is of such magnitude (of the order of 10^{-13}) that the sugars were probably without buffer effect in the present experiments. Regardless of the small amount of buffering that may have been contributed by these and by other substances such as flavonols and acid phosphates in the juice, or by slight ion-activity changes which may have occurred, citric acid and its basic salts, and perhaps some carbonate, play the chief rôle in this phenomenon. This is made evident by the fact that orange juice contains considerable amounts of inorganic salts, as represented by the percentages of ash in the juice (approximately 3.06 per cent. in Valencias and 3.27 per cent. in Navels, on a dry-weight basis). It is not known in what form these salts exist in the juice. Some of the cations undoubtedly are combined with the pectins, even in acid medium, and some are combined with the existing phosphate, sulphate, and other anions that are present; but most of the cations are probably combined with citric acid in the form of citrates. These inorganic citrates and citric acid are the sources of the substantial buffer capacity of orange juice.

The titration curve (fig. 12) for boiled orange juice was determined for the purpose of measuring the effect of heat on the buffer capacity of the juice. This type of experiment should show whether the heat decomposed or volatilized any of the acid constituents and thus lessened the amounts of NaOH required for neutralization. It should show, also, the effect of heat on the soluble pectins or on any other constituent that might hydrolyze and thus increase the amounts of NaOH required for neutralization. In the present experiments, the course of the titration curve for the orange juice that was boiled for 45 minutes (fig. 12, A), was similar to that for normal juice (fig. 12, B). The slightly higher pH value of the boiled juice showed that this juice required slightly less NaOH than unboiled juice for neutralization. Titration curves were also determined on juice boiled for 5, 10, 15, and 30 minutes, respectively; the values were so close to those of normal juice, however, that it was impractical to put them on the graph (fig. 12).

These results agree with those of DUNNE (**11**), who showed that boiled and filtered sap of wheat and buckwheat plants gave titration curves identical with those of the fresh sap; of BARNES (**4**), who observed that the hydrogen-ion concentration was only slightly lower in cooked apple juice than in fresh; and of MARSH (**16**), who showed that the buffer capacity of juice of nonacid vegetables was affected only slightly when heated in acid solution.

Summary and conclusions

The percentages of total soluble solids and of total sugars increased and that of acids decreased at approximately the same rate in the juice of Washington-Navel- and Valencia-orange fruits during maturation. After the fruits reached the stage of commercial maturity, the percentages of total soluble solids, total sugars, and reducing sugars in the juice continued to increase, while the sucrose and acids decreased, as the season advanced. The rate of increase in total soluble solids, however, was not so great as that of the total sugars and reducing sugars.

From September 29 (fruit still green) until March 2 (fruit well beyond the initial stage of maturity), the soluble solids of Washington-Navel-orange juice in California are composed of approximately 63 to 77 per cent. total sugars (35 to 40 per cent. sucrose and 27 to 38 per cent. reducing sugars), 23 to 8 per cent. acids, and 15 per cent. other substances. The values for Valencia juice of corresponding maturity are similar.

Where individual values for total soluble solids were plotted against the corresponding values for total sugars, noticeable scattering of the points occurred, but an increase in total soluble solids generally involved a corresponding increase in total sugars. This was verified by the high correlation coefficients calculated between total soluble solids and total sugars. A similar relation between total soluble solids and reducing sugars was noted, but a considerably greater scattering of the points occurred.

Although the total acids decreased during the growth and ripening of the fruit, while total soluble solids increased, large fluctuations in acid occurred without change in the total soluble solids and vice versa. Significant negative correlations were found to exist, however, between total soluble solids and total acids.

Due to the relatively high buffer capacity of the orange juice, large fluctuations in total acidity occurred without change in pH; but over a wide range of acid concentration (0.70 to 2.60 per cent.), the pH increased with a decrease in total acidity.

The experimental results show that the buffer capacity of orange juice is due chiefly to organic acids and inorganic salts.

The soluble pectins in the juice affected its buffer curve only slightly.

Boiling for 45 minutes, increased the initial pH of orange juice 0.2 over that of normal juice. This resulted in raising the buffer curve to a slightly higher pH level, but the shapes of the curves were the same.

Although the limits of dilution were not reached in these experiments, diluted (1:4) and undiluted juice had the same buffer capacity.

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MOLECULAR EQUIVALENCE OF CARBOHYDRATES TO CARBON DIOXIDE IN PHOTOSYNTHESIS

JAMES H. C. SMITH

Introduction

The photosynthetic reaction is commonly summarized by the following equation:

$$6\ CO_2 + 6\ H_2O = C_6H_{12}O_6 + 6\ O_2$$

The organic material formed has been commonly assumed to be glucose or some other carbohydrate. This equation is based principally on three types of facts: (a), the preponderance of carbohydrate material in plants, and the observed rapid increase in carbohydrates on illumination of green plants; (b), the near quantitative agreement of the increase in weight of organic matter produced and the amount of carbon dioxide photosynthesized; and (c), the approximation to the value of unity of the ratio between oxygen evolved and carbon dioxide absorbed.

The first of these facts, (a), is only qualitative and does not serve to establish the stoichiometric relations required by the equation. The investigations thus far reported, that have attempted to confirm the molecular equivalence between carbon dioxide assimilated and carbohydrate formed, have failed in their purpose as a brief review will show.

Fifty years ago, SAPOSCHNIKOFF (**12**) determined, as glucose, the amount of carbohydrate formed during photosynthesis in relation to the amount of carbon dioxide absorbed. In three experiments with sunflower leaves, he found that 63.8, 67.7 and 87.1 per cent. of the theoretical amount of carbohydrate was formed from the carbon dioxide absorbed. Because he found so great a discrepancy between the observed and theoretical recovery of carbohydrate, he concluded that "Bei allen drei Versuchen erhielt ich ein Deficit der Kohlenhydrate im Vergleich zu der zersetzten Kohlensäure. Auf diese Versuche hin muss man annehmen, dass ausser den Kohlenhydraten (Stärke) sich noch ein anderer Stoff bildet, und vielleicht ist dies Eiweissstoff."

Later KRASCHENINNIKOV (**8, 14b**) examined five different plants and found the following ratios: (A), the increase in dry weight as compared to the carbon dioxide absorbed; (B), the increase in carbohydrate in relation to the carbon dioxide absorbed; and (C), the increase in carbohydrate as related to the increase in dry weight.

	A	B	C
Bamboo	0.60	0.45	0.75
Cherry laurel	0.60	0.31	0.51
Sugar cane	0.67	0.50	0.75
Linden	0.75	0.56	0.75
Tobacco	0.65	0.37	0.57

From these results it can be seen that the average ratio of increase in dry weight to carbon dioxide absorbed, 0.654, closely approximates the theoretical value for a disaccharide, 0.648. This supports the supposition that the photosynthate is carbohydrate. Such close agreement may be misleading, however, for it may result from the accumulation of several products the sum of whose weights may equal fortuitously the weight of the hypothetical carbohydrate. The fact that the average increase in carbohydrates, reckoned as glucose, is only 64.2 per cent. of the amount required by theory, may be used to uphold the latter interpretation.

The ratio between the increase in dry weight of leaves of *Catalpa bignonioides* and the amount of carbon dioxide assimilated, was found by BROWN and ESCOMBE (**3**), using the half-leaf method of analysis, to be much larger than the increase expected on the assumption that the material synthesized was carbohydrate. This difference was ascribed to errors inherent in the method of determination.

In the most recent experiments, in which carbon isotopes have been used to estimate the amount of material formed, the results indicate that carbohydrate is not the only class of substance formed by photosynthetic action. The experiments carried out by RUBEN, HASSID, and KAMEN (**11**) on barley plants, using radioactive carbon, C^{11}, showed that only 25 per cent. of the carbon fixed by photosynthesis was water-soluble carbohydrate, and that not more than 10 per cent. could be contained in the insoluble material. From these experiments it was concluded that "The bulk of the radioactive material found in the plant is water soluble and is not carbohydrate. . . ." Recently heavy carbon, C^{13}, has been used for the investigation of the carbon metabolism of bean and radish plants. In an abstract of a paper given by BELKENGREN, NIER, and BURR (**2**), it is stated that "The conversion of newly formed photosynthate into chlorophyll, xanthophyll, lipids, cellulose, starch, protein, amino acids and amides has been measured." Whether or not the carbon first incorporated into the plant is in the form of diffusible carbohydrate was not stated.

The third type of observation that has led to the acceptance of the usual photosynthesis equation is the close approach to unity of the photosynthetic quotient, *i.e.*, the ratio of oxygen evolved to carbon dioxide absorbed. SPOEHR (**15**) has recently pointed out that even though the photosynthetic quotient were always equal to 1, this is no proof that the organic products formed consist solely of carbohydrates. So small a variation from unity as 3 per cent. as was found by MAQUENNE and DEMOUSSY (**10**), might indicate the formation of as much as 12 per cent. of protein. The fact that this ratio has been found in many cases to differ from unity by a considerable amount (**14a, 17**), and the fact that the quotient has been found to vary during the time course of photosynthesis (**6, 8**), also suggest that the process may not always conform to the equation cited, and that organic products other than carbohydrates may be synthesized during illumination.

From this short summary it is obvious that the facts are insufficient to

establish the widely accepted concept of the photosynthetic reaction, particularly the concept that carbohydrates are the exclusive organic products of photosynthesis. In order to gain more precise knowledge concerning the substances produced by the photosynthetic process, the quantitative relation between the amount of carbon dioxide absorbed and the nature and quantity of organic matter formed during photosynthesis should be investigated rigorously for a number of species of plants. As a contribution to this subject we have investigated this relation for sunflower leaves.

Experimentation

In these experiments the increase in carbon content of sunflower leaves, brought about by photosynthesis, was determined and compared with the corresponding increase in dry weight. The increase in carbon content was obtained by measuring the uptake of carbon dioxide during photosynthesis, and also by determining the gain in carbon by elementary analysis. Furthermore, the increase in various carbohydrate constituents of sunflower leaves was determined and related to the amount of carbon dioxide absorbed during illumination of the leaves.

Method of measuring carbon dioxide absorption

The amount of carbon dioxide taken up by the sunflower leaves was measured by determining the decrease in carbon dioxide concentration in a closed system. The arrangement was such that gas was circulated through the component parts of the apparatus in the following order: leaf chamber, gas pipet, pump, pH-measuring cell, and back to the leaf chamber again. The leaf chamber employed has already been described by Spoehr (**14c**). An "Autopulse Fuel Pump" reconstructed so as to be gas-tight, was used to circulate the gas within the system. The pH-measuring cell used in a previous investigation (**13**) was employed in this investigation. The cell contained a solution, 0.01 N with respect to sodium bicarbonate, and 1.0 N with respect to potassium chloride. The decrease in carbon dioxide concentration was determined from the change in pH of this solution, which was in equilibrium with the carbon dioxide in the circulating gas stream. A "Beckman pH Meter" was used to measure the pH of the bicarbonate solution. It was possible to read the pH accurately to 0.01 pH unit. The gas pipet, at 20° C., contained 28.604 mg. of carbon dioxide. It was fitted with two three-way stopcocks so that it could be cut out of the circulation system to be flushed and filled with pure carbon dioxide; then when desired, it could be cut into the circulation system so as to introduce a known volume of carbon dioxide. A by-pass around the gas pipet permitted circulation of the gas within the system without the gas passing through the pipet. Carbon dioxide for filling the pipet was taken from a commercial cylinder. The whole apparatus except the pump and some of the glass tubing, was immersed in a constant temperature bath, controlled either at 20.0° C. $\pm$ 0.1 or 10.0° $\pm$ 0.1. The walls of the water bath contained windows so that the leaf could be illuminated from the side.

After the leaf had been introduced into the leaf chamber, the apparatus was closed and the gas circulated within the system until the rate of respiration had become constant. The measured quantity of carbon dioxide was then introduced from the gas pipet. When equilibrium between the gas and the bicarbonate solution had been established (about 15 minutes was required for this), the leaf was illuminated by an approximately parallel beam from a 500-watt projection lamp. Removal of carbon dioxide by photosynthesis caused the pH to increase progressively. When approximately all of the added carbon dioxide had been used up, the light was turned off and the pH readings taken until a maximum value was reached, at which time the experiment was terminated. The amount of carbon dioxide absorbed by the leaf was calculated from the volume of the pipet, and the pH readings taken at the following times: when the carbon dioxide was introduced; when the light was turned on; and when the experiment was stopped.

Increased accuracy of measurement was obtained by arranging the experiment so that nearly the same quantity of carbon dioxide was absorbed as was pipetted into the apparatus. Under such conditions, the pH values obtained before introducing the carbon dioxide and after terminating the illumination were about equal. At this relatively high pH the small deviations in pH represented very small differences in carbon dioxide concentration. These differences were only a small fraction of the accurately measured quantity of carbon dioxide introduced, and any error introduced on account of inaccuracies in measuring them was entirely negligible. This technique made the method essentially a null-point method.

Methods of analysis

Determination of the ratio of the increase in dry weight to the amount of carbon dioxide absorbed.—In each of these experiments, the leaf which was used was taken from a plant which had been in the dark at least overnight. The petiole was cut from the stalk of the plant and immediately plunged into water. After rinsing the leaf with distilled water, the surface of the leaf was freed of adhering water. The halves of the leaf were then cut from the midrib and the areas outlined on sheets of heavy cellophane. The areas were measured by means of a planimeter. One half of the leaf was placed in a weighing bottle and dried at 85° to 90° C., first in a stream of air, and then in an ordinary drying oven. The other half of the leaf was fastened in a wire frame so as to hold the leaf flat and then placed in the leaf chamber of the photosynthesis apparatus.

The respiration of the leaf in the photosynthesis apparatus was measured so that proper correction could be made for the carbon lost by respiration. The correction had to be estimated for the period between the starting of the drying of the control sample and the beginning of the illumination of the photosynthesis sample. The correction usually amounted to about 2 or 3 per cent. of the amount of carbon dioxide absorbed.

When the measurement of the respiration was completed carbon dioxide was introduced into the photosynthesis apparatus, the light turned on, and photosynthesis carried out for the desired period. After the uptake of carbon dioxide had been measured (table I, col. 2), the leaf half was transferred to a weighing bottle and the dry weight determined exactly as described for the control.

The gain in carbon was also determined from elementary carbon analysis. The percentages of carbon in the dried material, from both the control sample (table I, col. 7) and photosynthesis sample (table I, col. 8) were obtained by the method of "Manometric Carbon Determination" described by VAN SLYKE and FOLCH (**18**). The weight of carbon in the control sample, corrected to a sample with an area equal to that of the photosynthesis

TABLE I

INCREASE IN DRY WEIGHT COMPARED WITH INCREASE IN CARBON ABSORBED DURING PHOTOSYNTHESIS

EXPERIMENT NO.	CARBON ABSORBED AS CO_2	INCREASE IN CARBON BY COMBUSTION	INCREASE IN DRY WEIGHT	△CARBON ABSORBED/△DRY WT.	△CARBON BY COMBUSTION/△DRY WT.	PERCENTAGE OF CARBON IN DRY WEIGHT OF SAMPLE	
						CONTROL	PHOTOSYNTHESIS
	mg.	*mg.*	*mg.*	%	%	%	%
1	5.278	5.223	12.9	40.9	40.5	46.00	45.58
2	7.560	8.907	20.6	36.7	43.2	44.75	44.98
3	7.302	8.037	17.2	42.5	46.7	45.30	45.48
4	6.783	6.466	17.6	38.5	36.7	47.00	45.50
5	6.025	6.222	14.1	42.7	44.1	46.12	45.90
6	6.186	5.554	14.0	44.2	39.7	45.25	44.67
		Average		40.9	41.8	45.74	45.35
		Probable error		± 0.9	± 1.1	± 0.24	± 0.18

sample, was subtracted from the amount of carbon in the photosynthesis sample. The difference was ascribed to carbon gained by photosynthesis. The values obtained in the various experiments are shown in table I, column 3. The wet combustion of leaf material had distinct advantage because the carbon in carbonates as well as organic carbon was determined quantitatively. This particular method was especially useful in the present investigation because of its speed and high degree of accuracy.

The gain in dry weight was obtained by subtracting the dry weight of the control sample, adjusted to an initial area equal to that of the photosynthesis sample, from the dry weight of the photosynthesis sample. The increases in dry weight obtained in different experiments are given in table I, column 4. The ratios of the gain in carbon, obtained from carbon dioxide absorption and from elementary carbon analysis, to gain in dry weight, are shown in table I, columns 5 and 6. It is evident that the ratios obtained by the two methods agree very well.

DETERMINATION OF THE RATIO OF THE INCREASE IN CARBOHYDRATES TO THE AMOUNT OF CARBON DIOXIDE ABSORBED.—In order to determine the increase in carbohydrates in relation to the amount of carbon dioxide absorbed, the following procedure was employed. The leaf used in each of the experiments was cut in the afternoon from a plant in the greenhouse. The petiole was plunged immediately into water. After a few minutes the leaf was washed with distilled water and kept overnight in a dark cabinet, the petiole being immersed in distilled water. (This technique is in contradistinction to the technique described in the preceding section in which the whole plant had been kept in darkness and the leaf was used for experimentation very shortly after being cut. The extended dark treatment in both cases was for the purpose of depleting the carbohydrate content of the leaf.) In the morning the halves of the leaf were cut from the midrib and each half cut in two transversally. The two diagonal quarters served for the control sample and the two remaining quarters for the photosynthesis sample. The leaf samples were placed in covered weighing bottles and weighed. The two quarters that were to be used for the control were spread out in the dark cabinet, with upper side down. At the time when illumination of the photosynthesis sample was begun, the control sample was removed from the dark cabinet and dropped into boiling 80 per cent. alcohol. By this procedure an equal period of respiration, prior to illumination, was secured for both control and photosynthesis samples.

The two quarters to be used for photosynthesis were fastened in a wire frame and brought into the photosynthesis apparatus. After respiration had become constant, the carbon dioxide was pipetted into the circulating gas stream. When equilibrium with the increased concentration of carbon dioxide had been established the light was turned on. Photosynthesis was allowed to continue until the carbon dioxide taken up by leaf approximately equaled the amount introduced, then the light was turned off. When the pH reached a maximum (in about 10 minutes at 20°, and 15 to 20 minutes at 10°), the experiment was stopped. The leaf was removed from the apparatus and immersed immediately in boiling 80 per cent. alcohol.

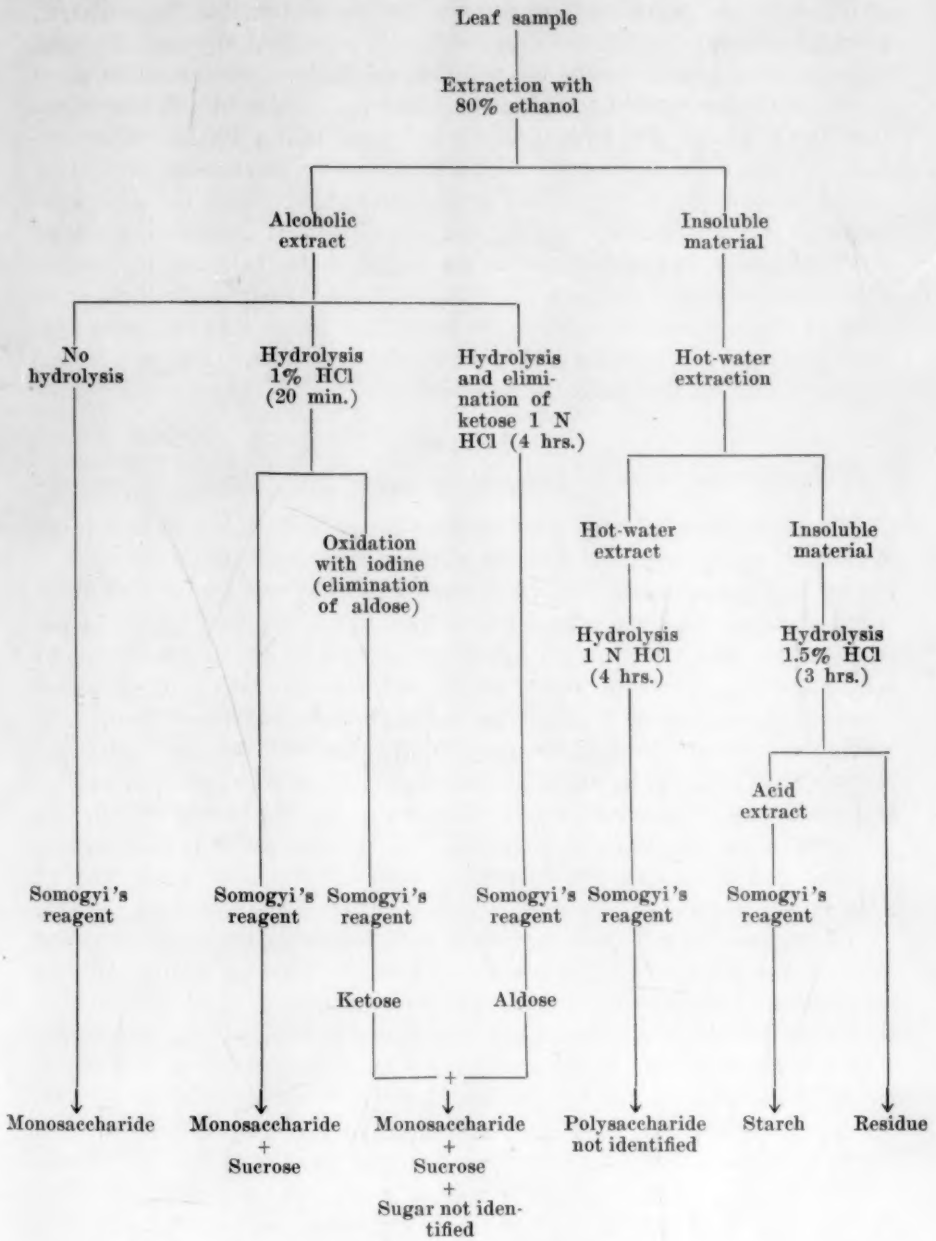
In some experiments at 20° an attempt was made to determine the fate of the new-formed carbohydrates immediately following their synthesis. For this purpose a prolonged period of respiration was introduced immediately following the short period of photosynthesis. At the close of the experiment, increases in the various fractions of carbohydrates and in the residue were determined and were correlated with the net gain in carbon absorbed as carbon dioxide. The term net gain is used to designate the difference between the total amount of carbon dioxide absorbed during photosynthesis minus the amount of carbon dioxide lost during the prolonged period of respiration.

In the photosynthesis experiments carried out at 10°, the control sample was placed in a refrigerator at about 5° for the period between weighing and immersion in the alcohol.

A synopsis of the procedure for carbohydrate analysis is given in the

form of a flow sheet. The various carbohydrates determined appear at the bottom of the diagram, and these are designated by the same terms that are used in the succeeding tables.

FLOW SHEET OF CARBOHYDRATE ANALYSIS



EXTRACTION.—After immersion in the alcohol the two samples were treated by as nearly identical procedures as possible. The leaf material was extracted five times with 20-ml. portions of boiling 80 per cent. ethanol. The alcoholic extracts after filtration were collected and analyzed for the dissolved sugars. The leaf material was then extracted four times with 20 ml. each time, of hot distilled water. The aqueous extracts were filtered, collected, and analyzed for the carbohydrates contained therein. The leaf material remaining from the hot-water extraction was suspended in about 20 ml. of 1.5 per cent. hydrochloric acid and hydrolyzed in a boiling water bath for 3 hours. The hydrolyzate was filtered into a 100-ml. volumetric flask. The leaf residue was extracted with hot water, three times with 20 ml. each, and once with 15 ml. These extracts were filtered into the same volumetric flask. The solution was diluted to volume and analyzed for sugar. Each extraction was carried out on the boiling water bath for a period of 20 minutes. The leaf material which had remained insoluble throughout all these extractions was collected in the same filter paper that had been used to filter each of the extracts, and was quantitatively transferred to a weighing bottle and the dry weight determined.

Analysis

ALCOHOLIC EXTRACT

The alcoholic extract was evaporated on the water bath to a small volume. Water (20 ml.) was added and the solution evaporated until the odor of alcohol had disappeared. The concentrated solution was transferred quantitatively to a centrifuge tube, 1 ml. of lead acetate solution (about 14 per cent.) added, and after a few minutes the precipitate was thrown down by centrifugation. The supernatant liquid was decanted into a 100-ml. volumetric flask containing 2 ml. of saturated sodium oxalate solution. The residue was washed three times by centrifugation with distilled water and the washings collected in the volumetric flask. After all of the lead oxalate had precipitated the solution was made to volume, thoroughly mixed, and the precipitate removed by filtration. In the filtrate, monosaccharides, sucrose, and a "sugar not identified" were determined by means of SOMOGYI's method (**1**). A reduction period of 25 minutes was employed (**7**).

The monosaccharides were estimated by determining the reducing power of the sugar solution without further treatment. Sucrose was determined by obtaining the increase in reducing sugar after a 20-minute hydrolysis with 1 per cent. hydrochloric acid. The hydrochloric acid was neutralized with sodium hydroxide using rosolic acid indicator and the sugars determined by reduction. The "sugar not identified" was determined by estimating the increase in reducing power brought about by hydrolysis with 1 N hydrochloric acid for 4 hours. The details of the method were as follows:

The sugar solution (5.00 ml.) was pipetted into a Pyrex test tube, 25×250 mm., and 0.46 ml. of concentrated hydrochloric acid added. The solution was hydrolyzed for 4 hours in the boiling water bath. After neu-

tralization of the acid with sodium hydroxide, the reducing power was determined.

To a duplicate sample, after hydrolysis, just enough solid sodium bicarbonate was added to neutralize the acid, the carbon dioxide evolved was removed by evacuation at the water pump, then 0.5 ml. of a buffer solution composed of potassium bicarbonate and potassium carbonate (*ca.* 2.5 N with respect to potassium ions, pH = 9.60) was introduced. This brought the solution to a pH of *ca.* 9.25, an optimal value for oxidizing glucose without appreciably attacking levulose. Iodine solution (0.3 ml. of 0.2 N) was introduced and the mixture allowed to stand for 60 minutes. After acidification with 0.3 ml. of 6 N hydrochloric acid, the liberated iodine was reduced with 2 per cent. sodium sulphite solution. The solution was neutralized with 6 N sodium hydroxide (rosolic acid indicator being used), 0.5 ml. of a glucose solution of known titre was added, and the reducing power of the solution was determined. By deducting the amount of added glucose, and estimate of the ketoses, presumably levulose, left undestroyed by the prolonged hydrolysis, was obtained. Subtraction of the amount of undestroyed levulose from the total amount of monosaccharide contained in the hydrolysis mixture provided an estimate of the total quantity of aldoses, herein

TABLE II

TABULAR PRESENTATION OF A SAMPLE EXPERIMENT. TEMPERATURE: 20.0° C.*

FRACTIONS	CONTROL	CONTROL CORR. FOR INITIAL WEIGHTS	PHOTOSYNTHESIS	INCREASE IN CARBOHYDRATE	INCREASE IN CARBON	RECOVERY OF CARBON ABSORBED
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	%
Initial fresh weight of sample	2661.7	.....	2691.2	.....	.....	.....
Carbon absorbed	.....	.....	.....	.....	7.871	.....
Monosaccharide	7.740	7.826	10.760	2.934	1.174	14.9
Sucrose	4.720	4.772	15.280	10.508	4.203	53.4
Sugar not identified	1.784	1.804	2.564	0.760	0.304	3.9
Polysaccharide not identified	9.780	9.889	10.550	0.661	0.264	3.4
Starch	17.840	18.038	22.640	4.602	1.841	23.4
Total soluble carbohydrate	.....	.....	.....	.....	7.786	98.9
Residue	70.9	71.7	74.5	2.80	1.244	15.8
Total recovery	.....	.....	.....	.....	9.030	114.7

* Explanation of table II. Col. 1: Designation of different fractions. Cols. 2 and 4: Weights of various fractions for the control, col. 2, and photosynthesis sample, col. 4; weights of carbohydrate fractions in terms of glucose, and actual weights of samples and residues. Col. 3: The values in col. 2 multiplied by the ratio of the initial fresh weights of photosynthesis sample to control sample. Col. 5: Values in col. 4 minus corresponding values in col. 3. Col. 6: The amount of carbon absorbed. Increase of carbon in each fraction: values for carbohydrates multiplied by 0.4000, the carbon content of glucose; value for residue multiplied by 0.4444, the carbon content of cellulose. Col. 7: The percentage of the carbon absorbed recovered in each fraction.

TABLE III

AMOUNT OF CARBOHYDRATES FORMED RELATIVE TO THE AMOUNT OF CARBON ABSORBED. TEMPERATURE: 20.0° C.*

EXPERIMENT NO. →	1	2	3	4	5	6	7	RECOVERY OF CARBON ABSORBED
MINUTES ILLUMINATION →	59	47	60	83	50	64	42	
	INCREASE IN CARBON							
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	%
Carbon absorbed	7.768	7.871	7.736	7.822	7.832	7.477	7.895	.....
Monosaccharides	0.260	1.174	1.263	0.718	0.860	0.826	0.326	10.0
Sucrose	4.110	4.203	3.966	4.839	4.591	2.926	3.609	51.8
Sugar not identified	0.389	0.304	0.256	0.168	0.617	0.000	−0.036	3.1
Polysaccharide not identified	−0.034	0.264	0.024	−0.174	−0.079	0.029	0.719	1.4
Starch	2.166	1.841	2.007	1.030	1.493	2.884	2.446	25.5
Total soluble carbohydrate	6.891	7.786	7.516	6.581	7.482	6.665	7.064	.....
Recovered as soluble carbohydrate: % of C absorbed	88.7	98.9	97.2	84.1	95.5	89.1	89.5	91.9 ± 1.5
Residue†	0.311	1.244	0.444	−0.444	0.711	0.800	0.471	6.5
Total carbon recovered	7.202	9.030	7.960	6.137	8.193	7.465	7.535	.....
Total recovery: % of C absorbed	92.7	114.7	102.9	78.5	104.6	99.8	95.4	98.4 ± 3.1

* Explanation of table III. Col. 1: Designation of the different fractions determined. Cols. 2 to 8: The amount of carbon absorbed. The increase of carbon in the different fractions. The values in these columns correspond to the values recorded in col. 6, table II. Col. 10: The percentage of the total carbon absorbed that was recovered in the different fractions.

† Carbon calculated from the increase in weight of the residue by multiplying by 0.4444, the percentage of carbon in cellulose.

designated glucose. The vigorous hydrolysis destroyed part of the glucose. By separate experiment this was found to be 4 per cent. [*Cf.* DAVIS and DAISH (5).] To obtain the true value for glucose the observed value was increased by 4 per cent.

In order to determine the total amount of levulose, the solution was heated in the boiling water bath for 20 minutes with 1 per cent. hydrochloric acid, and the reducing power determined after oxidation with iodine in the manner already described. The amount of levulose obtained by this analysis added to the total amount of glucose obtained by vigorous hydrolysis, yielded

TABLE IV

AMOUNT OF CARBOHYDRATES FORMED RELATIVE TO THE AMOUNT OF CARBON ABSORBED. TEMPERATURE: 10.0° C.*

EXPERIMENT NO. →	1	2	3	4	RECOVERY OF CARBON ABSORBED
MINUTES ILLUMINATION →	153	145	159	148	
	INCREASE IN CARBON				
	mg.	*mg.*	*mg.*	*mg.*	%
Carbon absorbed	8.050	8.081	8.064	8.073	.....
Monosaccharides	0.349	1.080	0.663	0.229	7.1
Sucrose	6.788	4.648	5.638	5.819	71.0
Sugar not identified	0.318	0.736	0.260	0.289	5.0
Polysaccharide not identified	−0.073	0.139	−0.521	0.292	−0.5
Starch	0.673	1.941	1.610	0.960	16.1
Total soluble carbohydrate	8.055	8.544	7.650	7.589	.....
Recovered as soluble carbohydrate: % of C absorbed	100.1	105.7	94.9	94.0	98.7 ± 2.1
Residue†	1.313	−0.298	1.848	−0.316	7.9
Total carbon recovered	9.368	8.246	9.498	7.273	.....
Total recovery: % of C absorbed	116.4	102.0	117.8	90.1	106.6 ± 5.0

* *Cf.* explanation of table III; see footnote (*) table III.
† From elementary carbon analyses.

the total amount of reducing sugars obtainable from this solution. From this total quantity was subtracted the amount of sugars obtained by mild hydrolysis, *viz.*, glucose and sucrose. The difference was designated, "sugar not identified."

Separate experiments demonstrated that analyses of mixtures of glucose and sucrose made by both the mild hydrolysis method and the vigorous hydrolysis method supplemented with the iodine oxidation, agreed on the average within 1.5 per cent.[1]

In summary, the sugars determined by the procedures described in this section were: monosaccharides, sucrose, and "sugar not identified."

[1] For a discussion of this type of methods for the determination of ketoses, *cf.* BROWNE and ZERBAN (4).

Hot-water extract

The solution obtained by extraction of the leaf material with hot water was concentrated to approximately 10 ml. on the boiling water bath. The concentrate was transferred quantitatively to a 50-ml. volumetric flask and diluted to the mark. After hydrolysis for 4 hours at 100° C. with 1 N hydrochloric acid, the reducing power was determined. The carbohydrate estimated in this way was designated "polysaccharide not identified."

Acid extract

The solution prepared by hydrolysis of the polysaccharides with 1.5 per cent. hydrochloric acid and extraction of the soluble sugars thus formed was neutralized and the reducing power of the solution determined. The carbohydrate determined in this manner was called starch.

In all determinations the analytical data for the control sample were adjusted to correspond to a sample with the initial weight of the photosynthesis sample. After making this adjustment, the difference in weights found in the corresponding categories of carbohydrates for the photosynthesis and the control samples was taken to be the increase in weight caused by photosynthesis. A typical experiment is detailed in table II. A summary of the increases of carbon in the carbohydrate and residue fractions correlated with the amount of carbon absorbed is given in tables III to V.

TABLE V

Correlation of carbohydrates recovered with the increase in carbon. Photosynthesis followed by prolonged respiration. Temperature: 20.0° C.*

Experiment no. →	1	2	Recovery of carbon: net gain
Minutes illumination →	84	57	
Minutes respiration after illumination →	196	243	
	Increase in carbon		
	mg.	*mg.*	%
Carbon absorbed: total	7.538	8.063	.....
Carbon absorbed: net gain	6.331	6.425	.....
Monosaccharides	2.202	2.249	34.9
Sucrose	2.766	2.964	44.9
Sugar not identified	0.091	0.564	5.1
Polysaccharide not identified	−0.026	−0.176	−1.6
Starch	1.149	1.153	18.1
Total soluble carbohydrate	6.182	6.754	.....
Recovered as soluble carbohydrate: % of net gain in carbon	97.6	105.1	101.4
Residue†	0.044	−0.088	−0.3
Total carbon recovered	6.226	6.666	.....
Total recovery: % of net gain in carbon	98.3	103.8	101.1

* For explanation of the significance of the columns, *cf.* table III, footnote (*).
† *Cf.* table III, footnote (†).

There was indication that pentoses or pentosans were present in the hot-water and the acid extracts. Even though these carbohydrates may have increased to a slight extent during photosynthesis, the percentage recovery of carbon in carbohydrates would have been little influenced by their increase because of the similarity in reducing power of the pentoses and glucose.

Discussion

From the ratio of the increase in carbon to the increase in dry weight, the percentage of carbon in the photosynthate was determined. From six experiments (table I) the percentage of carbon in the photosynthate was found to be 40.9 ± 0.9 per cent. when calculated from the amount of carbon dioxide absorbed. When reckoned from combustion analysis, it was found to be 41.8 ± 1.1 per cent. The average, 41.4 ± 0.6 per cent., approximates the percentage of carbon in a disaccharide, 42.10 per cent. This is indirect evidence that the photosynthate is carbohydrate in nature.

It may be of interest to note that the carbon percentage of the dried leaf portion used as control was found to be 45.74 ± 0.24 per cent. This value, when a reasonable correction for ash is made, would be raised to 51 or 52 per cent., which is considerably higher than the carbon content of the photosynthate, 41.4 per cent., or even of cellulose, 44.44 per cent. The causes for the accumulation in the leaf of compounds of higher carbon content than that of the photosynthate are not known. Two explanations of the effect may be suggested: (a) The photosynthate may be transformed into compounds of higher carbon content by metabolic processes of the leaf such as dehydration, cyclization, and reduction. (b) The photosynthate, although preponderantly carbohydrate may contain small quantities of materials of higher carbon content. Through preferential respiration, the carbohydrates may be used up. This would permit accumulation of the compounds of higher carbon content.

The nature and quantity of the organic matter formed was also determined by direct analysis of the leaf constituents. As it turned out, most of the constituents, which increased on illumination of the leaf, could be converted into substances which reduced alkaline copper reagent (SOMOGYI's reagent) and so were determined by this reagent. The increase in reducing power brought about by photosynthesis was calculated as glucose. An increase in the weight of the residual material, which remained insoluble throughout all the procedures employed, was also observed. This was a small part of the total increase. As yet the nature of this portion of the photosynthate is not known. It may be carbohydrate, *i.e.*, cellulose, hemicellulose, etc., or it may be protein inasmuch as the residue contained nitrogen.

In table VI are tabulated the increases observed in various classes of organic substances expressed as percentages of the carbon absorbed by the assimilation of a known amount of carbon dioxide. An examination of table VI shows that the recovery of carbon absorbed is very close to 100 per cent. Because so large a fraction of the assimilated carbon dioxide has been

TABLE VI

COMPARISON OF THE PROPORTIONS OF THE DIFFERENT CARBOHYDRATE FRACTIONS OBTAINED AT DIFFERENT TEMPERATURES

NUMBER OF EXPERIMENTS INCLUDED →	4	7
TEMPERATURE →	10.0°	20.0°
AVERAGE TIME OF ILLUMINATION: MINUTES →	156	58
	RECOVERY OF CARBON GAINED	
	%*	%†
Monosaccharide	7.1	10.0
Sucrose	71.0	51.8
Sugar not identified	5.0	3.1
Polysaccharide not identified	−0.5	1.4
Starch	16.1	25.5
Total soluble carbohydrate	98.7 ± 2.1	91.9 ± 1.5
Residue	7.9	6.5
Total recovery	106.6 ± 5.0	98.4 ± 3.1

* From table IV, col. 6.
† From table III, col. 9.

recovered as carbohydrate, especially at 10° C., it is apparent that the equivalence between carbon dioxide and carbohydrate required by the photosynthesis equation has in this instance been established.

While, on the average, the residues of the illuminated portions of the leaves showed an increase in weight, this was not always so. In fact, the variations were so great, especially at 10° C., as to cast doubt on the significance of the average increase observed.

Comparison of the results of the experiments carried out at 10° and 20° shows that at the lower temperature there was a greater recovery of carbon in sucrose and lesser recovery in starch and in monosaccharide. It is impossible to say at the present time whether this may be used as evidence to substantiate any of the alternative theories concerning the sugar first formed in photosynthesis.

If the assumption is made that carbohydrates are the sole products of photosynthesis (*cf.* table VI), then after a period of prolonged respiration the possibilities exist that the carbon recovered as carbohydrate will be less than, equal to, or greater than the net amount of carbon absorbed. If, during this period of respiration, the carbohydrates are transformed into other soluble substances the amount of carbohydrate recovered will be a smaller fraction of the net gain in carbon than if no respiration period had been allowed. If only carbohydrates are respired then the observed gain in carbohydrates should represent the same fraction of the net gain in carbon as when no respiration period was permitted. If, however, other organic substances are transformed into carbohydrates, or are respired in amounts comparable to the carbohydrates, then the possibility exists that the increase in carbohydrates will be greater with a long respiration period than without.

The results of the experiments were quite variable. Approximations to all three of these conditions were found among the various experiments performed, although recovery in excess of 100 per cent. was never greater than the probable variation. So far no generalization can be made concerning the types of reactions which occur in the absence of light. Because of the variability observed in the different leaf samples it is conceivable that the type of reaction depends on the character or condition of the individual leaf used in the experiment.

A striking feature of the prolonged respiration was the change brought about in the relative proportions of the different carbohydrates. For purposes of comparison, experiments employing long periods of respiration (table V) were chosen in which the total recovery of carbon was comparable to the recovery in the experiments with short respiration periods (table III). The comparison is shown in table VII.

TABLE VII

COMPARISON OF THE PROPORTIONS OF THE DIFFERENT CARBOHYDRATE FRACTIONS OBTAINED IN EXPERIMENTS WITH AND WITHOUT A PROLONGED RESPIRATION PERIOD FOLLOWING PHOTOSYNTHESIS. TEMPERATURE: 20.0° C.

NUMBER OF EXPERIMENTS INCLUDED →	2	7
	min.	*min.*
AVERAGE TIME OF ILLUMINATION →	71	58
AVERAGE TIME OF ILLUMINATION + RESPIRATION →	290	66
	RECOVERY OF CARBON GAINED	
	%*	%†
Monosaccharides	34.9	10.0
Sucrose	44.9	51.8
Sugar not identified	5.1	3.1
Polysaccharide not identified	−1.6	1.4
Starch	18.1	25.5
Total soluble carbohydrate	101.4	91.9
Residue	−0.3	6.5
Total recovery	101.1	98.4

* From table V, col. 4.
† From table III, col. 9.

From this comparison it is apparent that over the period of respiration the monosaccharides have increased to a very great degree. Starch and sucrose have both decreased. Possibly there is a significant decrease in the material contained in the insoluble residue, although this is doubtful in view of the large variations observed. It should be pointed out that under the conditions employed the leaves lost considerable water during photosynthesis and possessed a severe water deficit throughout the period of respiration. This may have influenced the change in the ratios of the different carbohydrates during the prolonged respiration period for it is well known that water deficit in sunflower leaves influences the dissolution of starch (**16**).

Mechanism of carbohydrate formation

From the results set forth in this paper it is evident that the ratio of the amount of carbohydrate formed to the amount of carbon dioxide absorbed is very close to the equivalence demanded by the commonly accepted photosynthesis equation. This close statistical correspondence, however, does not insure that the carbon atoms absorbed as carbon dioxide are the ones transformed into the carbohydrate molecules recovered. If comparison of different species of plants is justifiable, a correlation of the facts observed in this investigation with the facts already obtained by the use of radioactive carbon (**11**) might indicate that a direct transformation of carbon dioxide into carbohydrate is not accomplished by the photosynthetic process, otherwise a greater recovery of radioactive carbohydrates would have been obtained in the experiments with labeled carbon. To determine whether there is a direct conversion will require careful quantitative determinations to be carried out with labeled carbon on a number of different species of plants.

Conclusions and summary

From the results found in this investigation it may be stated that the equivalence demanded by the photosynthesis equation for the formation of carbohydrates from carbon dioxide has been demonstrated to be valid for sunflower leaves. There is a temperature effect on the proportion of the different classes of carbohydrates recovered after short periods of photosynthesis. This effect apparently favors the accumulation of disaccharides, such as sucrose, at the lower temperature.

The carbon content of the material accumulated during photosynthesis approaches that of a disaccharide. It is considerably lower than the average carbon content of the organic material of the leaf. This indicates that metabolic reactions subsequent to photosynthesis tend to convert the material photosynthesized into compounds of higher carbon content.

Respiration and transformation of the organic material formed during photosynthesis possibly follows different courses in different samples of sunflower leaves. In most cases, however, the principal material respired seems to be carbohydrate.

The results set forth in this paper have been obtained from the examination of sunflower leaves alone. Only after examination of a large number of species of plants will it be permissible to generalize more extensively.

The author wishes to acknowledge the many helpful suggestions received from his colleagues who were closely associated with this work: Dr. H. A. Spoehr, Dr. H. H. Strain, Dr. W. M. Manning, and Mr. Harold W. Milner. Special recognition is due Dr. Samuel S. Todd and Mr. David Frazier for their contribution to this research through making many of the analyses required.

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RÔLE OF ETHER SOLUBLE ORGANIC ACIDS IN THE CATION-ANION BALANCE IN PLANTS[1]

ELWOOD C. PIERCE AND C. O. APPLEMAN

(WITH SIX FIGURES)

Introduction

Factors that influence salt absorption by plants have long been a subject of study. Only recently, however, (**3, 4, 7, 8, 11, 16, 20, 23**), have the effects of certain metabolic processes in the plant been recognized as intimately connected with salt absorption and accumulation.

The present investigation has been concerned with the cation-anion balance in several species of crop plants with special reference to the rôle of ether soluble organic acids. Prior to the work of PUCHER, VICKERY, and WAKEMAN (**13, 14, 15**), on the quantitative determination of organic acids in plant material, lack of accurate methods had seriously curtailed investigation along these lines. A study has been made of the selective capacity of the various species with respect to inorganic ions; of the quantity and kinds of organic acids in the different species grown in similar culture medium; and of the correlation between inorganic cations and ether soluble organic acids in the different species as a group.

Materials and methods

CULTURE METHODS

Twelve species of plants were chosen for this investigation: spinach, variety Summer Savoy (*Spinacea oleracea* L.); beets, variety Early Superb (*Beta vulgaris* L.); wheat, variety Leapland (*Triticum aestivum* L.); Kentucky blue grass (*Poa pratensis* L.); alfalfa (*Medicago sativa* L.); lima bean (*Phaseolus limensis* Macf.); peas, variety Alaska (*Pisum sativum* L.); soybean, variety Biloxi (*Glycine soja* Sieb. and Zucc.); buckwheat (*Fagopyrum esculentum* Gaertn.); lettuce, variety Grand Rapids (*Lactuca sativa* L.); cantaloupe, variety White Seeded Pink Meat (*Cumcumis melo* L.); and tomato, variety Break of Day (*Lycopersicon esculentum* Mill.). The seeds of the various species were planted in sand and when the plants had attained sufficient height they were thinned to a few uniform plants per pot. Six pots were allotted to each species of plant.

The nutrient solution used had the following composition: 0.005 M Ca $(NO_3)_2$, 0.0025 M KH_2PO_4, and 0.0025 M $MgSO_4$. The elements Cu, Mn, Zn, B, and Fe were used in the usual small quantities to supplement the three main salts in the nutrient solution. This solution was applied to all plants at the rate of approximately 1 liter per crock, per 24 hours.

A modification of the SHIVE and STAHL capillary drip method (**19**) was

[1] Scientific Contribution no. 1840, Article no. A27, Department of Botany, University of Maryland Agricultural Experiment Station.

used to apply the nutrient solution. The modification consisted of a series of enamel basins, usually 3, connected by siphons and constantly supplied by solution from an inverted 18-liter bottle. The basins were placed on a shelf between twin rows of coffee urn liners filled with sand; these crocks permit good drainage. The shelf was 2 to 3 inches higher than the tops of the crocks. The basins were covered with round lids cut from Celotex and painted with asphalt paint to prevent water absorption. Four crocks were supplied with nutrient from each basin by inserting capillary tubes into notches cut on the under side of the lids. Adjustment of the capillary tubes was facilitated by adjustable metal supports fastened to the shelf near the basins. The inverted 18-liter bottle maintained a constant head of pressure and insured uniform delivery of nutrient solution at all times. After the initial adjustment of the capillaries, which was done with the aid of a stop-watch, the only thing required was to keep the 18-liter reservoir filled.

The 1940 crop was planted the latter part of September and all plants had been harvested by the end of November. In 1941 the experiment was repeated. This crop was planted the latter part of January and all plants had been harvested by the middle of April, with the exception of cantaloupe. The cantaloupe was not planted until April. The night temperature of the greenhouse was between 65° and 70° F.

These experiments were concerned only with the vegetative phase of growth. All plants were harvested before they blossomed or, in cases where blossoms appeared before sufficient vegetative growth had been attained, the blossoms were pinched off.

Tissue preparation and analytical methods

All samples were taken between 9:00 and 11:00 A.M. Each set of plants was divided into two equal lots and the fresh weight obtained immediately. Where possible both lots were divided into stems and leaves, petioles being included with the stems. The leaves and stems of one lot were placed in separate quart fruit jars, the lid put on tightly, and jars placed in a refrigerator at −15° C. The leaves and stems of the other lot were placed in separate wire baskets, dried in a hot air oven at 70° C., ground to pass a 60-mesh sieve, then stored in aluminum boxes until ready for analysis. The samples put up for juice analysis were frozen at −15° C. for at least 12 hours. After this time they were allowed to stand at room temperature until completely thawed. This usually required 3 to 4 hours. The material was then placed in a stout muslin cloth, subjected to 10,000 pounds pressure per square inch in a Carver hydraulic press, and the juice collected. The methods of the Association of Official Agricultural Chemists (1) were used for the final determination of calcium, magnesium, potassium, sulphur, and nitric acid.

Moisture.—Immediately after the plants were cut, duplicate 15–20 gram samples of leaves and stems were snipped into tared aluminum boxes. The samples were dried to constant weight in a vacuum oven at 65° C. and 2–3 cm. pressure. All results are based on dry tissue.

ASHING.—Duplicate ¾-gram samples of the dried, finely ground material were weighed into tared platinum crucibles. The material was moistened with 25 drops of sulphuric acid (1–10), dried in an oven at 95° C., then placed in a muffle furnace and ashed 4 hours at 600° C. The sulphuric acid was necessary to avoid explosion of the material while ashing.

CALCIUM.—The residue from the ash determination was used for the determination of calcium. The method used was identical with that employed for the analysis of calcium in the juice, except that the volume of the ash solution was 50 ml.

MAGNESIUM.—The filtrate and washings from the calcium determination did not contain sufficient magnesium to permit an accurate determination of this constituent. A larger aliquot, 20 ml., was used. This was treated in the same way as the calcium determination and magnesium determined on the filtrate and washings.

POTASSIUM.—Duplicate 10-ml. aliquots of the ash solution were used for the determination of potassium. The rapid official method (**1**) was used.

PHOSPHORUS AND SULPHUR.—A separate ashing was necessary for the determination of phosphorus and sulphur. Duplicate 1.0-gram samples were weighed into large porcelain crucibles and ashed by the magesium nitrate method (**1**). Phosphorus in the ash was determined by the method of FISKE and SUBBAROW (**6**). An Aminco photometer was used for the comparisons. A standardization curve was constructed, using varying concentrations of pure KH_2PO_4. Sulphur in the ash solution was determined by the official method (**1**).

TOTAL NON-VOLATILE ORGANIC ACIDS, OXALIC AND CITRIC ACIDS.—The methods used were those of PUCHER, VICKERY, and WAKEMAN (**13, 14**).

MALIC ACID.—The uranium acetate method of DUNBAR and BACON (**5**), modified by VICKERY and PUCHER (**21**) was used. Admittedly this method is not as good as the one developed later by PUCHER, VICKERY, and WAKEMAN (**15**) but the time saved justified its use.

NITRIC ACID.—The method used for the extraction of the organic acids also extracted the nitric acid quantitatively. A suitable aliquot was reduced, distilled, and Nesslerized (**1**). Readings were taken in an Aminco photometer and the quantity of nitrogen read from a standardization curve.

SAP ANALYSIS

The determination of sap soluble constituents in the extracted juice is based on the work of SAYRE and MORRIS (**17, 18**).

TOTAL SOLIDS.—Duplicate 2-ml. samples of the juice were dried in a hot air oven at 70° C., then placed in a vacuum oven at 65° C. and 2 to 3 cm. pressure until constant weights were obtained.

OXALIC ACID.—Aliquots of 5 to 10 ml. of juice, depending upon the oxalic acid content, were pipetted into 25-ml. volumetric flasks and made to volume. Duplicate 10-ml. aliquots of the diluted juice were measured into 100-ml. beakers and 0.5 normal HCl added until slightly acid to Congo red. This

was allowed to stand overnight and any material that flocculated was filtered off on an asbestos mat. The coagulum was washed 2 or 3 times with small amounts of water slightly acidified with HCl. From this point the method was identical with that used by PUCHER, VICKERY, and WAKEMAN (**13**) to determine oxalic acid in the ether extract of dried tissue.

CALCIUM.—Duplicate 10-ml. samples of the juice were measured into small porcelain crucibles and dried in a hot air oven at 70° C. The material

TABLE I

INORGANIC CATIONS, 1940 CROP

PLANT		MILLIEQUIVALENTS PER 100 GRAMS OF DRY TISSUE				
		TOTAL MAGNESIUM	TOTAL POTASSIUM	TOTAL CALCIUM	SOLUBLE CALCIUM	SOLUBLE CALCIUM, PERCENTAGE OF TOTAL
		m. eq.	*m. eq.*	*m. eq.*	*m. eq.*	%
Lima bean	Leaves	74.0	111.2	117.2	80.5	68.7
	Stems	64.6	167.3	81.7	57.5	70.5
Peas	Leaves	52.6	78.9	128.0	.....	.....
	Stems	34.5	115.1	59.0	.....	.....
Alfalfa	Leaves	56.7	80.6	132.6	69.6	52.5
	Stems	42.8	92.7	45.6	16.3	35.8
Soybean	Leaves	93.5	88.5	112.3	55.8	49.7
	Stems	85.5	146.1	80.3	38.8	48.3
Beets	Leaves	240.6	176.6	99.0	2.3	2.4
	Petioles	92.9	348.5	27.8	4.7	17.2
Spinach	Leaves	165.3	186.5	127.6	1.3	1.0
	Petioles	103.0	333.7	74.3	2.0	2.8
Buckwheat	Leaves	188.5	61.9	146.6	4.8	3.3
	Stems	110.8	243.0	105.6	13.6	12.9
Bluegrass	Leaves	52.6	155.1	24.6	20.3	82.6
Wheat	Leaves	52.1	191.5	22.6	17.0	75.3
Tomato	Leaves	114.3	102.0	183.0	91.0	49.8
	Stems	129.4	228.0	104.3	33.8	32.4
Lettuce	Leaves	59.2	216.9	81.6	48.7	59.6
Cantaloupe	Leaves	125.0	96.3	291.3	45.7	15.7
	Stems	93.2	216.9	79.6	46.0	57.8

was moistened with a few drops of sulphuric acid (1:10) and heated gently over a low flame until thoroughly charred. The crucibles were then placed in a muffle furnace and ashed for 4 hours at 600° C. The addition of sulphuric acid was necessary to eliminate explosion of the material during ashing.

The ash was dissolved in dilute HCl, taken to dryness to dehydrate any silica, redissolved in dilute HCl and made to a volume of 25 ml. Calcium was determined on 10-ml. aliquots of this solution by the official micro method (**1**).

Results

The plants from the 1940 and 1941 crops were quite comparable with respect to age, height, and fresh weight, with the exception of cantaloupe.

The 1940 crop of cantaloupe was grown in the late fall when light intensity was low and consequently there was less growth than in the 1941 crop which was grown in April and May when light intensity was increasing. Regardless of the difference in the amount of growth of the two crops, analyses based on dry weight showed them to be quite comparable. The absolute values for age, height, and fresh weight are not recorded as they were of importance only in comparing the growth of the two crops.

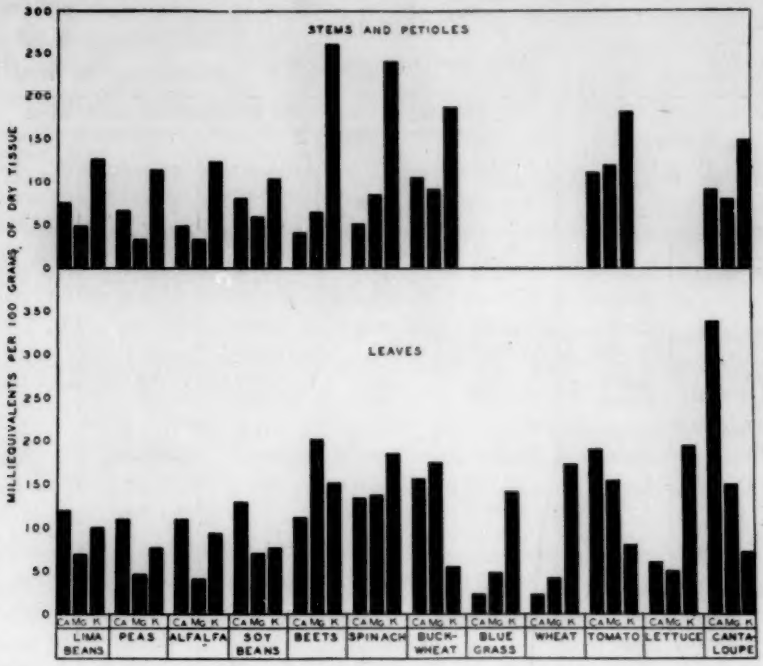

FIG. 1. Distribution of Ca, Mg, and K in leaves and stems of the species tested (1941 crop).

INORGANIC CATIONS AND ANIONS

Table I gives the inorganic cations in milliequivalents per 100 grams of dry tissue in the different plants for the 1940 crop and table II the inorganic cations and anions for the 1941 crop. The inorganic anions were not determined in the 1940 crop. It can be seen that the inorganic ion content is quite comparable in the two crops. The data on the cations of the 1941 crop are shown more clearly in fiigure 1. Figure 2 shows graphically the distribution of calcium. Since the 1940 crop was similar it is not included.

It is clearly shown that when different species of plants are all grown under similar conditions of nutrient supply they take up inorganic ions in varying proportions according to inherent characteristics of the plant. It should be noted that even plants in the same families all tend to accumulate cations in the same relative proportions (legumes: soy beans, lima beans, alfalfa, and peas; the Chenopodiaceae: beets and spinach; the grasses: bluegrass and wheat. These results corroborate those of NEWTON (9).

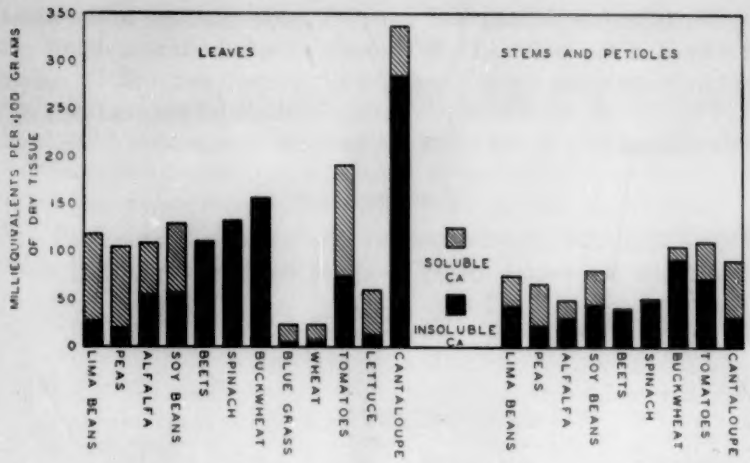

FIG. 2. Relative amounts of soluble and insoluble calcium in leaves and stems of the species tested (1941 crop).

Calcium and magnesium tend to accumulate in greater quantities in the leaf blade tissue than in the stems and petioles. The reverse is true of potas-

TABLE II

INORGANIC ANIONS AND CATIONS, 1941 CROP

PLANT		MILLIEQUIVALENTS PER 100 GRAMS OF DRY TISSUE							SOLUBLE CALCIUM, PERCENTAGE OF TOTAL
		TOTAL NO^-_3	TOTAL $SO^=_4$	TOTAL H_2PO_4	TOTAL MAGNESIUM	TOTAL POTASSIUM	TOTAL CALCIUM	SOLUBLE CALCIUM	
		m. eq.	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	%
Lima bean	Leaves	28.4	18.5	13.1	69.6	98.9	118.3	90.8	76.8
	Stems	32.3	9.5	15.2	48.5	125.7	75.0	31.5	42.1
Peas	Leaves	19.5	25.9	23.9	47.8	77.8	105.5	86.5	82.1
	Stems	48.7	12.8	15.3	33.7	114.4	66.0	43.1	65.3
Alfalfa	Leaves	24.6	34.4	19.4	41.4	93.4	109.0	53.9	49.4
	Stems	29.6	15.1	21.8	33.4	123.6	49.7	18.4	37.3
Soybean	Leaves	15.0	23.0	24.9	71.0	76.6	128.7	71.6	55.7
	Stems	32.1	23.3	23.6	58.1	104.2	81.0	36.0	44.4
Beets	Leaves	22.3	30.0	24.9	201.8	151.7	112.0	1.3	1.2
	Petioles	157.5	12.4	15.2	65.3	261.0	42.3	2.7	8.9
Spinach	Leaves	40.4	30.5	39.7	137.3	186.7	133.7	1.1	0.9
	Petioles	100.3	22.2	37.9	85.2	242.1	51.3	2.6	5.2
Buckwheat	Leaves	17.7	21.0	27.1	174.9	57.8	157.0	2.8	1.8
	Stems	162.5	12.2	22.1	92.1	186.7	106.0	13.5	12.7
Bluegrass	Leaves	74.3	26.7	23.4	47.7	142.2	23.0	17.0	75.7
Wheat	Leaves	76.9	30.0	35.7	41.7	173.1	22.7	15.0	66.7
Tomato	Leaves	20.4	91.8	30.0	105.3	82.4	191.7	116.8	60.9
	Stems	106.4	15.2	29.7	119.3	181.4	112.0	39.7	35.3
Lettuce	Leaves	54.3	24.8	26.3	50.5	195.2	60.7	47.5	78.5
Cantaloupe	Leaves	44.3	72.4	26.9	148.6	72.6	336.7	51.0	15.2
	Stems	120.7	27.4	26.1	81.1	148.0	91.7	59.8	65.5

sium. Much larger quantities of this element accumulate in the stems and petioles than in the leaf blades. The exceedingly high amount of calcium present in cantaloupe leaves is of special interest and will be considered later. The soluble calcium as percentage of the total ranges from 70 to 80 per cent. in some plants and down to 1 per cent. in others.

Organic acids

Tables III and IV give the values of the various organic acids in milliequivalents per 100 grams of dry tissue in the two crops, 1940 and 1941.

TABLE III

Organic acids, 1940 crop

Plant		Milliequivalents per 100 grams of dry tissue					Unknown as percentage of total
		Total acids	Oxalic	Malic	Citric	Unknown acids	
		m. eq.	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	%
Lima bean	Leaves	237.2	30.2	24.9	38.5	143.6	60.5
	Stems	259.3	29.6	30.2	32.2	167.3	64.5
Peas	Leaves	255.7	17.0	56.1	86.0	96.6	37.8
	Stems	219.6	6.0	47.0	56.0	110.6	50.4
Alfalfa	Leaves	199.5	30.0	24.9	22.9	121.7	61.0
	Stems	166.8	9.8	16.8	10.9	129.3	77.5
Soybean	Leaves	246.1	17.4	1.3	58.0	169.4	68.8
	Stems	235.5	21.8	26.2	20.6	166.9	70.9
Beets	Leaves	405.9	298.0	21.2	20.6	66.1	16.3
	Petioles	201.8	86.6	40.0	22.4	52.8	26.2
Spinach	Leaves	362.1	280.4	6.5	3.7	71.5	19.7
	Petioles	238.5	150.9	16.8	4.9	65.9	27.6
Buckwheat	Leaves	353.6	253.8	15.8	26.4	57.6	16.3
	Stems	207.2	109.2	16.8	13.4	67.8	32.7
Bluegrass	Leaves	142.7	0.0	7.7	26.3	108.7	76.2
Wheat	Leaves	133.5	0.0	16.4	6.5	110.6	82.8
Tomato	Leaves	239.7	69.3	30.9	53.8	85.7	35.7
	Stems	220.5	82.1	52.7	10.7	75.0	34.0
Lettuce	Leaves	197.2	2.3	72.1	18.6	104.2	52.8
Cantaloupe	Leaves	85.6	0.0	22.2	34.0	29.4	34.3
	Stems	129.7	0.0	53.7	10.7	65.3	50.3

A word of warning is necessary when comparing the data in this experiment with data from plants grown under different conditions. As Clark (**4**) and Wadleigh and Shive (**23**) have shown and as Vickery and Pucher (**22**) have stated, the kinds and amounts of organic acids occurring in plants are profoundly influenced by the form in which nitrogen is supplied to the plant.

It is evident that in many cases it is possible to account for only a small amount of the total organic acidity as oxalic, malic, and citric acids. In wheat and blue grass the unknown organic acids make up 70 to 80 per cent. of the total; in beets, spinach, and buckwheat 70 to 85 per cent. of the total acids can be accounted for. Data of this nature clearly show that there is

still much to be done in the way of developing suitable methods for the determination of the acids of the unknown group.

CATION-ANION BALANCE

PUCHER, VICKERY, and WAKEMAN (**16**) have shown in tobacco grown under controlled fertilizer conditions a large excess of positive ions over inorganic anions, and this excess is balanced by the ether soluble organic acids. An attempt has been made in the present investigation to determine if this is a common phenomenon among plants in general.

TABLE IV

ORGANIC ACIDS, 1941 CROP

PLANT		MILLIEQUIVALENTS PER 100 GRAMS OF DRY TISSUE					UNKNOWN AS PERCENTAGE OF TOTAL
		TOTAL ACIDS	OXALIC	MALIC	CITRIC	UNKNOWN ACIDS	
		m. eq.	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	%
Lima bean	Leaves	249.7	16.9	65.8	48.6	118.4	47.4
	Stems	171.1	31.1	24.5	33.1	82.4	48.2
Peas	Leaves	212.9	15.1	42.6	80.8	74.4	34.9
	Stems	181.0	7.3	46.5	34.5	92.7	51.2
Alfalfa	Leaves	185.7	14.1	21.8	14.5	135.3	72.9
	Stems	140.7	4.9	30.9	8.4	96.5	68.6
Soybean	Leaves	248.7	15.9	10.6	104.6	117.6	47.3
	Stems	177.0	18.9	10.9	26.8	120.4	68.0
Beets	Leaves	427.5	322.9	13.4	32.0	59.2	13.8
	Petioles	203.1	97.8	28.9	24.8	51.6	25.4
Spinach	Leaves	380.4	309.0	8.9	10.5	52.0	13.7
	Petioles	226.2	110.2	60.1	9.6	46.3	20.5
Buckwheat	Leaves	360.1	265.7	14.1	13.8	66.5	18.5
	Stems	205.6	110.4	34.1	6.6	54.5	26.5
Bluegrass	Leaves	108.5	0.0	9.1	27.8	71.6	66.0
Wheat	Leaves	122.1	0.0	18.3	9.6	94.2	77.2
Tomato	Leaves	269.8	45.2	61.1	74.4	89.1	33.0
	Stems	231.6	81.3	89.3	12.3	48.7	21.0
Lettuce	Leaves	220.8	1.4	109.0	32.2	78.2	35.4
Cantaloupe	Leaves	99.2	0.0	26.2	19.4	53.6	54.0
	Stems	119.4	0.0	54.4	5.9	59.1	49.5

In the analyses no attempt was made to fractionate organic and inorganic sulphur and phosphorus. In the calculations of milliequivalents of anions all sulphur was considered as sulphate and all phosphorus as diacid phosphate. This is not strictly true, as phosphorus and sulphur are known to enter into many organic compounds as proteins, phosphatides, hexose phosphates, etc. It seems safe to assume, however, that the major portions of these elements are in the inorganic form in the plant. All phosphorus was calculated as the monophosphate ion as this is practically the only form in which it can exist at the pH characteristic of the sap of the plants tested.

Table V presents data showing the relationship between the excess inorganic cations and ether soluble organic acids in the leaves and stems. Figure 3 presents the data of table V in graphic form.

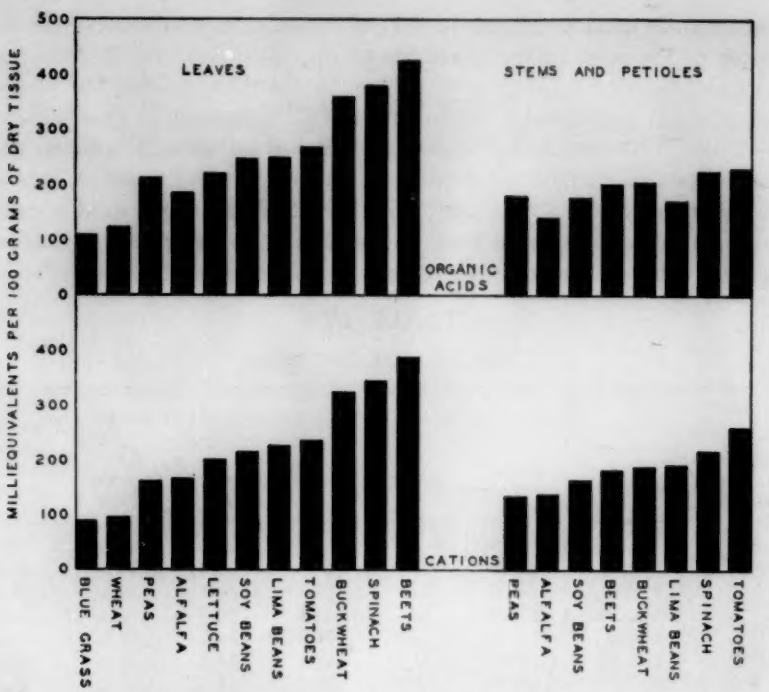

FIG. 3. Correlation between excess cations and total organic acids in leaves and stems of several species (1941 crop).

As far as the authors are aware these are the first data to show that inorganic cations and ether soluble organic acids are positively correlated in

TABLE V

CORRELATION BETWEEN EXCESS INORGANIC CATIONS AND TOTAL ORGANIC ACIDS, 1941 CROP

PLANT	MILLIEQUIVALENTS PER 100 GRAMS OF DRY TISSUE							
	LEAVES				STEMS AND PETIOLES			
	TOTAL Ca, Mg, K	TOTAL ANIONS*	EXCESS CATIONS†	TOTAL ORGANIC ACIDS	TOTAL Ca, Mg, K	TOTAL ANIONS*	EXCESS CATIONS†	TOTAL ORGANIC ACIDS
	m. eq.	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*
Lima bean	286.8	59.9	226.9	249.7	249.2	57.0	192.2	171.1
Peas	231.2	69.2	162.0	212.9	214.1	76.9	137.2	181.0
Alfalfa	243.8	78.4	165.4	185.7	205.7	66.5	139.2	140.7
Soybean	276.2	62.9	213.3	248.7	243.3	79.0	164.3	177.0
Beets	465.5	77.2	388.3	427.5	368.5	185.0	183.5	203.1
Spinach	457.7	110.5	347.2	380.4	378.7	160.4	218.3	226.2
Buckwheat	389.7	65.8	323.9	360.1	384.8	196.8	188.0	205.6
Bluegrass	212.9	124.3	88.6	108.5	.....	.....	.....	.....
Wheat	237.0	142.6	94.7	122.1	.....	.....	.....	.....
Tomato	379.2	142.1	237.1	269.8	412.7	151.3	261.4	231.6
Lettuce	306.3	105.4	200.9	220.8	.....	.....	.....	.....
Cantaloupe	557.9	143.6	414.3	99.2	320.8	174.2	146.6	119.4

* NO^{-}_{3}, $SO^{=}_{4}$, $H_2PO^{-}_{4}$.
† Total cations minus inorganic anions.

a series of different species of plants. The correlation in the leaf blade tissue is exceedingly high (+0.996), that in the stem and petiole tissue not as high (+0.798).

It will be noted in table V, that the cantaloupe plant has the highest cation content, but the lowest organic acid content, of any of the species tested. The exceedingly high amount of calcium present in the leaves of cantaloupe is rather unusual. Approximately 85 per cent. is insoluble but

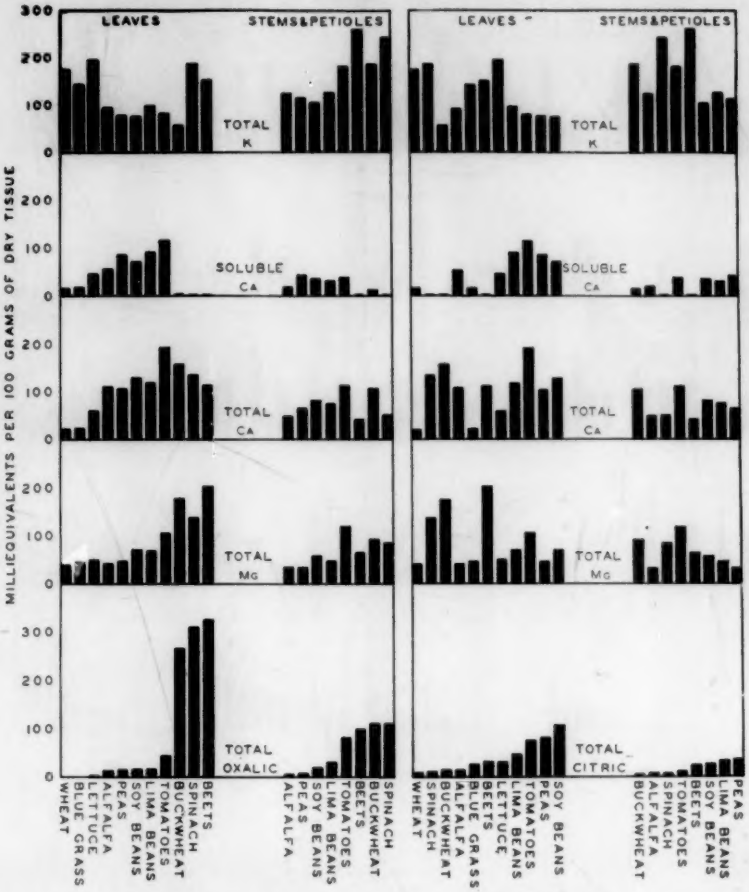

FIG. 4. Comparison of the amounts of oxalic and citric acid with the amounts of different cations occurring in the plants tested.

it is not obvious in what combination it exists. There is no oxalic acid in the cantaloupe plant so the possibility of insoluble calcium oxalate is excluded. The pH 7.4 to 7.6 of the expressed sap is unusually high. This immediately suggests the possibility that part of the cations are tied up as carbonate and bicarbonate. Preliminary experiments, however, do not indicate a very large amount of CO_2 in the sap. Under more careful conditions this may not prove to be the case. Work is now in progress to deter-

mine what substances other than phosphate, sulphate, nitrate, and organic acids are binding the large quantities of cations in this plant. ILJIN (8) found, in general, that soluble calcium in plants was positively correlated with malic and citric acid content. PIATNITSKY (11) found that excised leaves of adult tobacco plants accumulated citric acid when exposed to a solution of $MgCl_2$. The increase in citric acid was nearly equivalent to the decrease in malic acid.

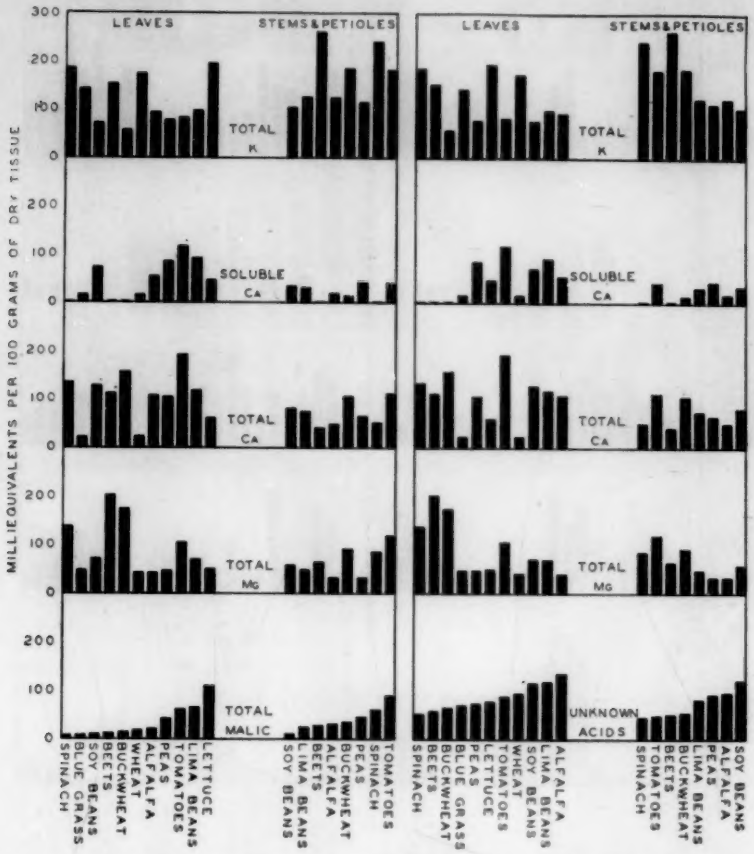

FIG. 5. Comparison of the amounts of malic acid and acids of the unknown group with the amounts of different cations occurring in the plants tested (1941 crop).

Figures 4 and 5 compare graphically the amounts of determined organic acids as well as the unknown group with the amounts of cations found in the various plants. No striking correlations are evident. Malic and citric acids are found to be somewhat correlated with soluble calcium, particularly in the leaf tissue. Citric acid and those acids of the unknown group show a small negative correlation with total magnesium content in the stems and petioles.

In spinach, beets, and buckwheat a relatively large proportion of the

organic acids could be accounted for principally as oxalic acid. Figure 4 shows that in the leaves of these plants total calcium decreases as total oxalic acid increases. Total magnesium content is considerably higher in these plants than in the others, indicating enhanced magnesium absorption.

Results of the present investigation indicate that those plants which produce no oxalic acid have very small quantities of insoluble calcium (figures 4 and 6). The major portion of the calcium is in a sap soluble state. There are exceptions to this, such as cantaloupe. In the plants that produce some oxalic acid, but no large quantity, all of the oxalic acid is precipitated, presumably as calcium oxalate. The major portion of the calcium, however, is

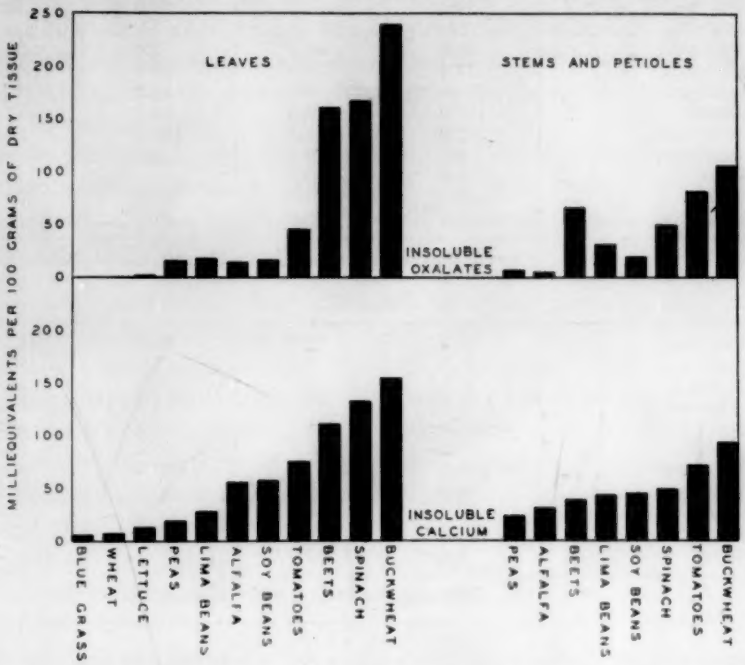

FIG. 6. Relationship between insoluble calcium and insoluble oxalates in leaves and stems of several species tested (1941 crop).

still in a sap soluble state. Those plants (beets, spinach, and buckwheat), which produce large quantities of oxalic acid have practically all of the calcium in an insoluble state, only traces of sap soluble calcium occurring. In addition these plants have considerable quantities of oxalates dissolved in the cell sap. According to this, the picture ranges from those plants with no oxalic acid and a relatively large proportion of sap soluble calcium to those plants with large quantities of oxalic acid and little or no soluble calcium.

Table VI shows the high positive correlation between insoluble calcium and insoluble oxalates. Figure 6 presents the same data in graphic form for the 1941 crop. It will be noted that in the three plants producing the larg-

TABLE VI

CORRELATION BETWEEN INSOLUBLE OXALIC ACID AND INSOLUBLE CALCIUM, 1940 CROP

PLANT	MILLIEQUIVALENTS PER 100 GRAMS OF DRY TISSUE			
	LEAVES		STEMS AND PETIOLES	
	INSOLUBLE OXALIC ACID	INSOLUBLE CALCIUM	INSOLUBLE OXALIC ACID	INSOLUBLE CALCIUM
	m. eq.	*m. eq.*	*m. eq.*	*m. eq.*
Lima bean	30.2	36.7	29.6	24.2
Peas	.....	.....	.....	.....
Alfalfa	30.0	63.0	9.8	29.3
Soybean	17.4	56.5	21.8	41.5
Beets	209.8	96.7	37.9	23.1
Spinach	155.0	126.3	84.3	72.3
Buckwheat	242.9	141.8	109.2	92.0
Bluegrass	0.0	4.3	.....	.....
Wheat	0.0	5.6	.....	.....
Tomato	69.3	92.0	82.1	70.5
Lettuce	2.3	32.9	.....	.....
	Correlation coefficient = + .886		Correlation coefficient = + .929	
	1941 CROP			
Lima bean	16.9	27.5	31.1	43.5
Peas	51.1	19.0	7.5	22.9
Alfalfa	14.1	55.1	4.9	31.3
Soybean	15.9	57.1	18.9	45.0
Beets	161.0	110.7	66.1	39.6
Spinach	167.4	132.6	49.4	48.7
Buckwheat	239.8	154.2	100.6	92.5
Bluegrass	0.0	6.0	.....	.....
Wheat	0.0	7.7	.....	.....
Tomato	45.2	74.9	81.3	72.3
Lettuce	1.4	13.2	.....	.....
	Correlation coefficient = + .946		Correlation coefficient = + .884	

est quantities of oxalic acid, insoluble oxalates exceed the amount of insoluble calcium. Obviously some other cation besides calcium must exist in the form of an insoluble oxalate in the plant. Whenever juice of beets, spinach, or buckwheat is allowed to stand for a few hours a white crystalline precipitate appears. This has been identified as pure magnesium oxalate. Previous evidence has been obtained by the senior author (**12**) that magnesium is precipitated by oxalic acid. Magnesium oxalate is known to form a supersaturated solution rather easily and it appears that this is what happens in the plant. With an increased uptake of magnesium, however, the limits of supersaturation are bound to be exceeded and some of the magnesium oxalate will be precipitated.

Summary and conclusions

Twelve different species of plants were grown in the greenhouse under controlled solution culture and all plants received the same nutrient supply.

Chemical analyses were made with the idea of studying the cation-anion balance in the different species of plants.

Inorganic ions were found to be taken up in varying proportions according to inherent characteristics of the plant. Plants in the same family tended to accumulate ions in relatively the same proportions.

Data were obtained on the kinds and amounts of organic acids occurring in a variety of plants. In some plants the unknown fraction of organic acids made up 70 to 80 per cent. of the total, while in others the unknown fraction amounted to only 15 to 25 per cent.

In all of the plants studied, except cantaloupe, a large excess of inorganic cations over inorganic anions was found and when all plants were considered together this excess was found to be highly correlated with total ether soluble organic acids. Cantaloupe was the outstanding exception. It contained the largest amounts of cations but the smallest amounts of organic acids of any of the species tested.

In the leaves malic and citric acids showed a rather low positive correlation with soluble calcium. Citric acid and those acids of the unknown group showed a rather small negative correlation with total magnesium content in the stems and petioles.

Insoluble oxalates and insoluble calcium were found to be highly correlated when the plants were considered as a group. In three cases insoluble oxalic acid exceeded the amount of insoluble calcium. Evidence was cited that the additional insoluble oxalic acid was present as magnesium oxalate. Magnesium content increased with increased oxalic acid content.

Those plants with little or no oxalic acid had a large proportion of the calcium in a sap soluble state, while those plants high in oxalic acid had but traces of sap soluble calcium.

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AMOUNT AND DURATION OF GROWTH OF VARIOUS SPECIES OF TREE SEEDLINGS

PAUL J. KRAMER

(WITH FOUR FIGURES)

Introduction

This paper deals with the results of measurements of the length of growing season and amount of growth made by tree seedlings of eleven different species, including some species with northern and some with southern ranges. The seedlings were grown together for three years under the same environmental conditions. The species used were balsam fir (*Abies balsamea* (L.) Mill.), loblolly pine (*Pinus taeda* L.), shortleaf pine (*P. echinata* Mill.), slash pine (*P. caribaea* Morelet), red pine (*P. resinosa* Ait.), white pine (*P. strobus* L.), white ash (*Fraxinus americana* L.), eastern red oak (*Quercus borealis maxima* (Marsh) Ashe), white oak (*Q. alba* L.), yellow poplar (*Liriodendron tulipifera* L.) and black walnut (*Juglans nigra* L.). The balsam fir, red, and white pine were obtained from the New York State College of Forestry at Syracuse and were three years old. The red and white oak were grown from seed in our greenhouses and were one year old; all of the other species were one-year-old stock from the state nursery at Clayton, North Carolina.

The red pine and balsam fir do not naturally extend as far south as North Carolina; and white pine, with a few exceptions, is restricted to the mountains in this region. The normal range of slash pine does not extend as far north as North Carolina, while loblolly and shortleaf pine occur both north and south of North Carolina. This combination of species gave an excellent opportunity to compare the behavior of conifers planted north and south of their natural ranges with that of conifers growing well inside their natural ranges. Durham is within the southeastern limit of distribution for black walnut and red oak, and the other three deciduous species extend from the Gulf to Canada; thus all of the deciduous species used in this experiment were within their natural range.

A number of studies of tree growth have been made in America but none of them include comparisons of most of the species dealt with in this study, and none of them include data on the growth of northern species when transplanted outside their normal range. No formal review of the literature will be made as the pertinent papers are cited in connection with various phases of the work. BALDWIN (1) has discussed most of the important papers.

Methods

Thirty trees of each species were planted in a rectangular plot of ground in rows two feet apart, the individual trees being 18 inches apart in the rows. The individuals of each species except black walnut were located at random

in the plot so that all species were exposed to any variations in soil moisture and fertility in different parts of the plot. The black walnut was obtained too late to be included in the randomized planting so it was planted in a row along one side of the plot and only 25 trees were grown. Stakes were driven into the ground beside each tree the first year and reference marks made by driving nails through the stakes at the ground level. The second and third years' measurements were made from small nails driven into the trees. Measurements were made to 0.5 cm. with a meter stick. At the end of the second growing season the white ash and yellow poplar were so large that they were beginning to shade the other species so they were removed.

Results

The results of this experiment are discussed under three headings: first, the relative amounts of shoot growth made by the various species in a growing season; second, the relative lengths of growing seasons of the various species; and third, a comparison of the length of growing season of certain species at Durham, North Carolina, and in New England.

Relative growth

Relative amounts of growth made by the various species in a season are shown graphically in figures 1 and 2. As would be expected there are very considerable species differences. Yellow poplar and white ash grew much more rapidly than red and white oak and black walnut. The third season black walnut grew somewhat more rapidly than the oaks. The white ash and yellow poplar had become so large by the end of the second season that they seriously shaded the smaller conifers so they were cut. Differences in growth rate between the coniferous species were also consistently large. Loblolly pine made the most growth every year, slash and shortleaf pine made about two-thirds as much growth as loblolly; red and white pine made only about one-sixth as much growth as loblolly pine. The behavior of the pines in this experiment is in accord with their behavior in experimental plantations on the Duke Forest (**14**). Eight years after planting, the average heights of trees in adjacent plantations set out the same winter were: loblolly pine, 18.1; slash pine, 12.6; red pine, 2.3; and white pine, 3.8 feet. These trees were in adjacent plantations and their rate of growth might have been somewhat affected by variations in soil conditions; the trees in our experiment, however, were randomized among each other so that all species were exposed to similar soil conditions.

There were considerable differences in mortality among the seedlings of the various species, especially among the conifers (table I). The only deciduous species with high mortality was black walnut, 64 per cent. of the seedlings of this species having died by the end of the third year. Only three to ten per cent. of the seedlings of the other deciduous species died. Black walnut is rather difficult to transplant because of its large root system and furthermore it was not randomized with the other species, so one end

of the row may have been in relatively less favorable soil, thus increasing its mortality. The mortality among loblolly, shortleaf, and slash pine was only three to ten per cent., but 26 per cent. of the red pine and 46 per cent. of the white pine died during the three-year period. The highest mortality was in balsam fir of which 80 per cent. died the first season and 93 per cent. were dead by the end of the third season. In another nearby plot planted with seedlings from the same sources, and in most respects a replication of the one described in this paper, the mortality of red and white pine and balsam fir was comparable.

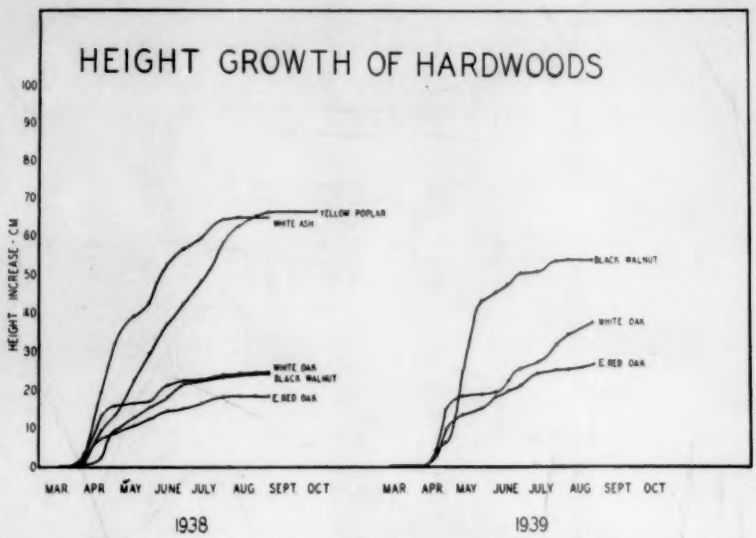

FIG. 1. Height growth of hardwoods during second and third seasons after planting. White ash and yellow poplar were removed at end of second season because they were overtopping other species.

LENGTH OF GROWING SEASON

Large differences existed between species with respect to length of growing season and course of growth during a season as well as with respect to the amount of growth made in a season. These data are summarized in table I. Figure 1 shows graphically the behavior of the hardwood species. All species resumed growth somewhat later the third season than the second season. Inspection of climatological data for Durham indicates that March, 1938, was about 6° F. warmer than average and March, 1939, about 3° F. warmer than average; it is doubtful, however, if this small difference in temperature can explain the difference in time of resumption of growth. Rainfall was adequate both springs. While white ash and yellow poplar behaved similarly in making more growth than any of the other hardwoods the manner in which this growth was distributed over the growing season was quite different in the two species (see figures 1 and 3). Both species resumed growth about April 1, but white ash made 50 per cent. of its growth

TABLE I

GROWTH MADE EACH MONTH EXPRESSED AS PERCENTAGE OF TOTAL SEASONS GROWTH. MORTALITY EXPRESSED AS PERCENTAGE OF ORIGINAL NUMBER OF SEEDLINGS DEAD AT END OF EACH SEASON

Month	White ash	Yellow poplar	E. Red oak		White oak		Black walnut		Loblolly pine		Shortleaf pine		Slash pine		Red pine		White pine		Balsam fir
	1938	1938	1938	1939	1938	1939	1938	1939	1938	1939	1938	1939	1938	1939	1938	1939	1938	1939	1938
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
March	0.0	0.0	4.0	0.0	6.0	0.0	2.0	0.0	5.0	2.0	2.0	3.0	6.0	4.0	16.0	8.0	13.0	0.0	0.0
April	49.0	20.0	45.0	45.0	57.0	45.0	38.0	18.0	21.0	14.0	24.0	16.0	22.0	19.0	66.0	55.0	67.0	65.0	37.0
May	20.0	25.0	21.0	17.0	6.0	5.0	26.0	65.0	15.0	27.0	13.0	22.0	14.0	25.0	8.0	32.0	10.0	32.0	47.0
June	20.0	21.0	15.0	21.0	21.0	20.0	24.0	11.0	21.0	23.0	17.0	19.0	17.0	17.0	5.0	2.0	4.0	3.0	3.0
July	11.0	23.0	15.0	12.0	7.0	20.0	7.0	6.0	15.0	14.0	16.0	14.0	17.0	14.0	3.0	0.0	6.0	0.0	12.0
August	1.0	10.0	0.0	5.0	3.0	10.0	3.0	0.0	14.0	13.0	20.0	16.0	16.0	12.0	2.0	3.0	0.0	0.0	0.0
September	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	5.0	6.0	7.0	8.0	7.0	7.0	0.0	0.0	0.0	0.0	0.0
October	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	1.0	1.0	2.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0
Growing season in days	130	160	125	135	140	135	150	115	210	210	200	200	200	200	140	150	127	115	120
Mortality	0.0	6.6	10.0	10.0	3.3	3.3	36.0	64.0	3.3	6.6	3.3	3.3	10.0	10.0	3.3	26.6	20.0	46.6	80.0

in April, slowed down in May and June, and ceased in late July or early August after a growing period of about 130 days. Yellow poplar had no peak month, but made about 20 per cent. of its growth each month from April to July inclusive, slowed down in August, and ceased in September after a growing period of 160 days. Red and white oak most nearly resembled white ash because they made about half the season's growth in April and had shorter growing seasons than yellow poplar. Even these species did not behave in exactly the same manner, however. Growth of eastern red oak gradually slowed down after the April peak and ceased in August while white oak had a peak in April, grew but little in May, but made rapid growth in June, then gradually slowed down and ceased growth in August. Compared to the oaks, black walnut started rather slowly in the spring, reached a peak in late April or May, and ceased growth late in July or in August

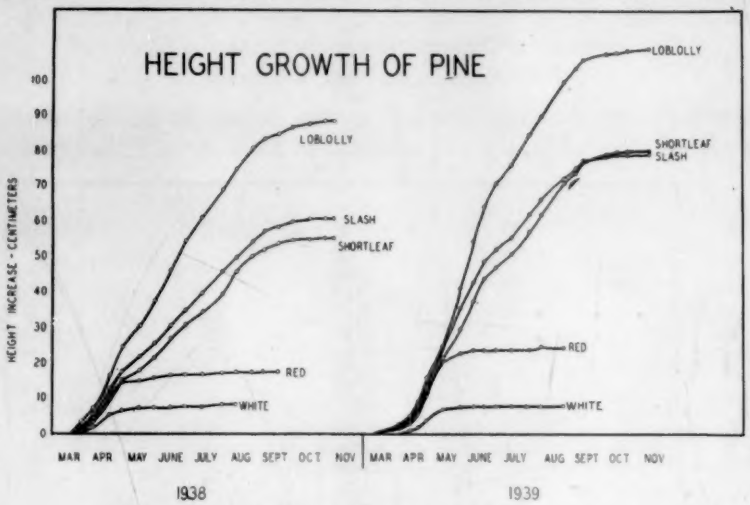

FIG. 2. Height growth of pines during two seasons.

after a growing season of 115 or more days. Individual trees ceased growth at intervals during the growing season, and even formed buds, then after a few weeks resumed growth again. This variation in the behavior of individual trees was most noticeable among the oaks, but occurred in all species, including the conifers.

The behavior of the pines is of particular interest because it provided a comparison of the length of growing season of the northern species, when grown in the south, with the southern species. The data for pine are shown graphically in figure 2. In general the northern species of pines made slightly more growth in March than the southern species though white pine did not resume growth until April 1 in 1939. The few surviving balsam fir seedlings resumed growth 7 to 10 days later than any other species studied. Both red and white pine made over 65 per cent. of their growth in April and over 90 per cent. of their growth by the end of May. White pine ceased

growth in late July while red pine made a small amount of growth in August. In contrast to the northern species, which made most of their growth in April and completely ceased to grow by July or August, loblolly, slash, and shortleaf pine had no well-defined peak in growth. All three species made measurable growth in March and made from 15 to 20 per cent. of the season's growth in every month from April to August, six to eight per cent. in September, and finally ceased growth early in October after growing seasons of 200 to 210 days. REED (**17**) in an earlier experiment at Durham, North Carolina, reported that loblolly pine resumed growth in late March and shortleaf pine in early April. Both species ceased growth about September 8, after a growing season of 170 days for loblolly, and 155 days for shortleaf pine. REED's trees were six years old and over six feet high and this difference in size may have some bearing on the difference in length of growing season.

COMPARISON OF LENGTH OF GROWING SEASON IN NORTH CAROLINA AND NEW ENGLAND

Few data are available concerning the growth of hardwoods farther north except those of ILLICK (**7**) obtained at Mont Alto, Pennsylvania, and

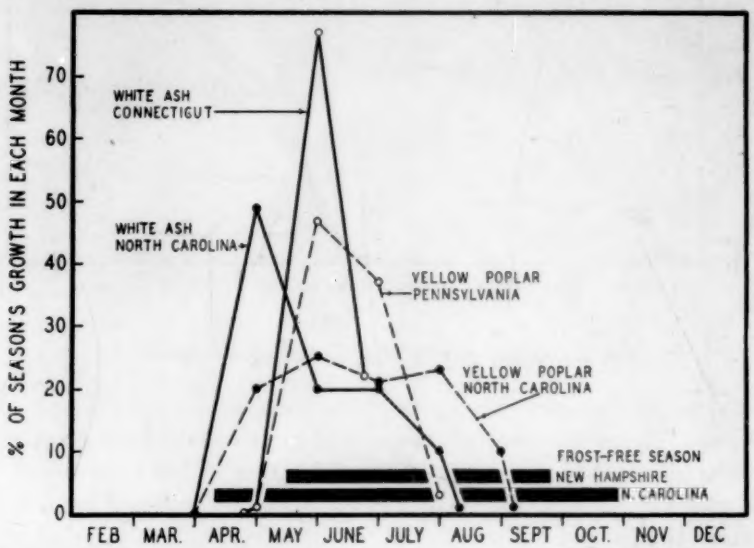

FIG. 3. Height growth of white ash and yellow poplar at Durham, North Carolina, and at northern stations. The data for white ash in Connecticut are from KIENHOLZ (**11**); the data for yellow poplar in Pennsylvania are from ILLICK (**7**).

those obtained by KIENHOLZ (**11**) in northwestern Connecticut. Their observations were made on trees which were older and larger than those measured in this study. Age and size of tree may have some effect on the length of the growing season but no definite information is available concerning the relation between length of growing season and age of tree. Figure 3 shows the length of growing season for white ash in North Carolina

and Connecticut and the length of growing season for yellow poplar in North Carolina and Pennsylvania as plotted from the data of KIENHOLZ and ILLICK. Study of figure 3 shows that, as would be expected, white ash and yellow poplar resumed growth nearly a month later at the northern stations and also ceased growth several weeks sooner. The time of maximum growth was also shifted about a month later at the more northern stations and the percentage of the season's growth made during the first month after the resumption of growth was much higher at the northern stations. The data for red oak give curves very similar to those for white ash at both stations. White ash and red oak grew twice as long in North Carolina as in Connecticut, and yellow poplar grew much longer in North Carolina than in Pennsylvania or Connecticut. It seems probable that the 95-day growing period

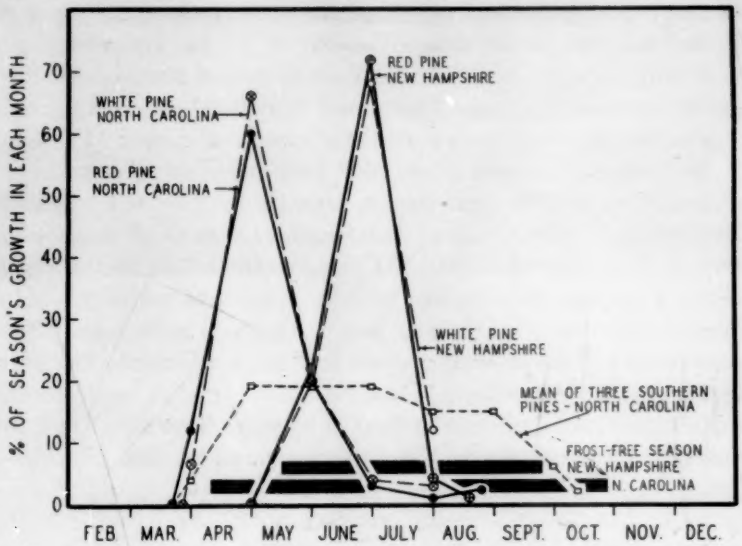

FIG. 4. Height growth of red and white pine at Durham, North Carolina, and Keene, New Hampshire. The New Hampshire data are from KIENHOLZ (10).

reported by ILLICK (7) in Pennsylvania does not include some late summer growth as KIENHOLZ (11) indicates that the growing season of yellow poplar in Connecticut is 105 to 110 days. JOHNSTON (9) reported that black, white, and post oak in the Missouri Ozarks had a growing season of only 19 days and made 90 per cent. of their growth in eleven days between April 27 and May 7. Those trees which averaged eight years in age and four feet in height behaved very differently from the oak in Connecticut and North Carolina. Our three- or four-year-old white oak seedlings grew from late March to late July and made about 50 per cent. of their total growth in April.

Several studies of the length of growing season of conifers have been made in various sections of the United States, but the most useful data for comparison seem to be those of KIENHOLZ (10), obtained in Southern New Hampshire. Figure 4 shows growth curves for red and white pine at Dur-

ham, North Carolina, and Keene, New Hampshire. Growth of both species started over a month later in New Hampshire and the peak came two months later than in North Carolina. The data of TRYON and FINN (**20**) from southern New York and FRIESNER (**6**) from southern Indiana indicate behavior at those places intermediate between that in North Carolina and in New Hampshire. Since the growing season naturally starts later and ends sooner at the northern stations than in North Carolina the growing season for red pine decreases from 145 days in North Carolina to 110 days in southern New Hampshire and the growing season of white pine decreases from 120 days in North Carolina to 100 days in New Hampshire. The 70-day growing season reported by ILLICK (**7**) for white pine in Pennsylvania seems abnormally short and probably does not include the small amount of growth made during the last month of the growing season. COOK (**4**) reported that red pine in Rensselaer County, N. Y., has an average growing season of only 53 days, but this also evidently does not include the small amount of late growth because TRYON and FINN (**20**) report a growing season of over 100 days only a short distance away. BALDWIN (**1**) found that white pine resumed growth about the same time at Ithaca, New York, Petersham, Massachusetts, and Berlin, New Hampshire, but was somewhat later at Cupsuptic Lake, Maine. The length of growing seasons seemed to be about the same, approximately 100 days, at all stations, including the one in Maine. FRIESNER (**6**) reported growth of red and white pine after the midsummer cessation of growth as late as October in Indiana. This possibly was related to an unusually warm and rainy autumn. The only data available on the other species of pine are those obtained by JOHNSTON (**9**) in the Missouri Ozarks where shortleaf pine grew from mid-April to early September, a total of 140 to 150 days compared to 200 days in North Carolina.

Discussion

Studies of tree growth under various environmental conditions should contribute to our knowledge of the factors determining the length of growing season and amount of growth made in a season. If we could obtain a more complete understanding of the factors determining the length of growing season and the time of maximum growth we might be able to control these factors advantageously in growing trees of better quality more rapidly. Two groups of factors interact in determining the amount of growth made by any tree. These are: (1), the hereditary potentialities as determined by its genetic composition; and (2), the complex of environmental factors to which the tree is exposed. The environment affects the amount and quality of growth by affecting the internal processes and conditions such as photosynthesis, respiration, accumulation of food, and water balance, within the limits of the hereditary potentialities of the individual tree or the species.

Inspection of figures 1 and 2 shows the very large genetic differences between species as expressed by differences in amount of growth and length of

growing season of seedlings grown in the same environment. Even so drastic a change in environment as resulted from transferring red and white pine from New England to North Carolina merely increased the absolute lengths of their growing seasons, but did not change the relative lengths nor the shapes of their growth curves (fig. 4). White ash and yellow poplar have longer growing seasons in North Carolina than in Pennsylvania or Connecticut but the essential differences between their growth curves exist in both environments.

These results serve to emphasize that the length of growing season and course of shoot growth of trees is relatively independent of normal fluctuations in environmental factors during the growing season. Severe droughts or excessively low temperatures may check growth, but the usual variations in moisture and temperature have little effect. COOK (**4**) reported that only unusual deficiency in rainfall retarded the growth of conifers and FRIESNER (**6**) reported that heavy rainfall in June did not check the rapid decrease in rate of growth of pines which occurred during that month. REED and MACDOUGAL (**18**) state that periodicity in the growth of the orange tree cannot be ascribed to environmental factors or depletion of reserve food. Apparently the length of growing season and shape of the growth curve is determined more by internal genetic factors than by the environment. It is generally agreed that shoot growth is principally made at the expense of stored food rather than from the products of current photosynthesis (**3, 16**). BURGER (**2**) concluded that the amount of any one year's shoot growth is largely dependent on the supply of reserve food accumulated the previous season, and hence depends on the weather of the previous season more than on the weather of the season in which growth is being measured. If this is true it helps to explain the relative independence of shoot growth from external conditions during that season. Presumably there must be species differences in the amount of food accumulated and the rate at which this is digested and translocated in the spring to the growing stem tips. This might explain the difference in seasonal distribution of growth existing, for example, between white ash and yellow poplar or between the northern and southern pines. It seems possible that trees with long growing seasons might not only utilize reserve food, but later in the same season they might also utilize the products of current photosynthesis in shoot growth as well as in cambial activity, thus prolonging their growing season beyond that of species which depend chiefly or entirely on reserve food. There appears to be no definite relation between length of growing season and amount of shoot growth. White ash has a short period of growth and yellow poplar a long period, but both make about the same amount of growth. While loblolly pine seedlings make considerably more growth than slash and shortleaf pine, their growing season is only a few days longer and this could not explain the difference in amount of growth.

Another feature of the growing season of many tree species to which attention should be called is the relatively small portion of the frost-free

season in which most of the shoot growth occurs. This was emphasized by ILLICK (7) who reported that at Mont Alto, Pennsylvania, 90 per cent. of all shoot growth occurs in 40 days, or about 25 per cent. of the frost-free season, and most species cease shoot growth in June. Ten species growing at Durham, North Carolina, had an average growing season of 160 days, or 80 per cent. of the frost-free season and made 90 per cent. of their growth in 100 days, or 50 per cent. of the frost-free period. The five hardwood species had an average growing season of 140 days or only 60 per cent. of the frost-free season and made over half of the season's growth in about 30 per cent. of the frost-free season. The average growing season of the species measured by KIENHOLZ in Connecticut and New Hampshire was 65 to 70 per cent. of the frost-free season; those trees, like the trees in North Carolina, averaged 90 per cent. of their growth in about 50 per cent. of the frost-free period. Apparently very few tree species in eastern North America have a growing season equal to the frost-free season, the only such species known to the writer being loblolly, slash, and shortleaf pine observed in this study, and eastern larch observed by KIENHOLZ (11). Even these species ceased growth two or three weeks before the average date of the first killing frost. It was possible for them to have a growing season equal in length to the frost-free season only because they had started growth before the average date of the last killing frost in the spring. Not only do most trees cease shoot growth several weeks or even months before the first frosts of autumn, but they also resume growth in the spring before the danger of frost is over. This is not only true in North Carolina but also in Pennsylvania, New England, and probably all over the northern hemisphere. BALDWIN (1) mentions severe frost injury in New England; DAY and PEACE (5) report that it is common in England and it is mentioned by various continental writers (3).

This puzzling situation constitutes one of the most interesting problems in tree physiology. Why should most trees resume shoot growth in the spring before the danger of frost is past, then cease growth in midsummer or at least weeks before the first killing frost of autumn? Cessation of growth in midsummer does not have any obvious survival value to the species, but would rather serve to decrease its ability to compete with species having longer growing seasons. Of course, if shoot growth continued until the first frost the succulent tips might be killed, but there is no reason to suppose that occasional injury of this sort would be any more serious than the injury caused by spring frosts. The writer has caused yellow poplar, loblolly, and slash pine to grow until frost by exposing them to a long photoperiod and although some stem tips were killed the growth of the trees the following season was not measurably less than that of uninjured trees.

Well over half a century ago SACHS (19) observed that growth frequently ceased while temperature and other conditions favored vegetative activity and was resumed under conditions which appeared to be far less favorable. He stated that while the dormant period occurred at a definite season and therefore appeared to be dependent on temperature and moisture, it really in his opinion, depended chiefly on changes occurring within the plant. It

is clear that while increasing soil and air temperatures are important factors in determining the time of resumption of growth in the spring, decreasing soil and air temperatures certainly do not determine the time of cessation of growth, because growth of most species ceases before air and soil temperature begins to decrease. Severe droughts might prematurely stop growth, but ordinary fluctuations in rainfall do not, nor can they explain why some species cease growth long before others. KLEBS (**12**) in one of the earlier discussions of the periodicity of tree growth suggested that temporary depletion of minerals might cause the cessation of growth by tropical trees, but this would scarcely explain the difference in behavior of different species in the same habitat. He also suggested that length of day may be an important factor in determining the length of the growing season, although he seems to have been thinking in terms of increased photosynthesis rather than photoperiod. JESTER and KRAMER (**8**) observed that seedlings of a number of species of trees, both in the greenhouse and out-of-doors, grew much later in the season, when given a photoperiod as long as that of midsummer by use of supplementary electric lights, than did controls exposed to the normally decreasing photoperiod of late summer and autumn.

The species used in the present study were also planted in a nearby plot illuminated each evening after the days began to shorten so that they always received a photoperiod as long as that of midsummer. Yellow poplar, loblolly pine and slash pine showed definite responses, growing about three weeks later under the lights than without them. Yellow poplar continued to form new leaves until frost and slash pine showed considerable winter killing of stem tips, probably because the new growth did not have time to harden before the first frost. There is no doubt that the length of growing season of many species can be affected by the photoperiod under experimental conditions, but the growing season of some species is not affected. It is probable that photoperiod affects the growing season of some species in nature, but there is no clear evidence that it is the only or even the major factor controlling the time at which trees cease growth.

Obviously more information must be collected before we can explain the differences in growth habits of trees. While considerable data are available on the relation between growth and environmental conditions, more might profitably be collected. It would be particularly valuable to have more data on the response of seedlings to variations in environmental factors under controlled or at least partly controlled conditions of soil and air temperature, soil moisture, and photoperiod. Much more information concerning the physiology of species with long and short growing seasons is also needed, with special reference to food accumulation and its subsequent utilization in growth.

Summary

Eleven species of tree seedlings were grown together in the same plot for three years at Durham, North Carolina, and their growth measured at intervals through the growing season.

Of the five hardwoods, white ash and yellow poplar grew most rapidly, making over twice as much growth as red and white oak, and black walnut. Loblolly pine grew more rapidly than any other of the six conifers. Shortleaf and slash pine made about two-thirds the growth of loblolly, but the northern species, red and white pine and balsam fir, made only one-sixth the growth made by loblolly pine.

There were also considerable differences between species with respect to length of growing season and time of maximum growth. All species resumed growth in late March or early April. White ash, eastern red oak, white oak, and black walnut made about 50 per cent. of their growth by May 1 and ceased growth in July or early August after growing seasons of 130 to 135 days. Yellow poplar had no peak month, but grew about the same amount every month from April through July and finally ceased growth in September after a growing season of 160 days.

Loblolly, shortleaf, and slash pine behaved alike, starting growth in late March, making 15 to 20 per cent. of their total growth each month from April through August, and finally ceasing growth in October. Red and white pine, which are characteristic of more northern latitudes and higher altitudes, resumed growth very little sooner than the southern species but made 65 per cent. of their growth by May first, 90 per cent. by June first, and ceased growth in July or August after a growing season of 120 to 145 days, compared to 200 days or more for the southern species.

Trees growing in North Carolina uniformly had longer growing seasons than the same species in New England, but most of the growth occurred at the beginning of the season and the trees did not benefit by the longer growing season. Red and white pine and balsam fir made less growth and had a higher mortality than would have been expected in New England. The seasonal course of growth of a species was the same in North Carolina as in New England. The shape of the growth curve is apparently determined largely by internal factors rather than environmental factors.

Most tree species for which data are available behave alike, both in New England and North Carolina, in that they all resume growth in the spring before danger of frost is past but most of them cease growth in mid or late summer, long before the first frost of autumn. As a result many species use only one-half to three-fourths of the frost-free season.

While decreasing photoperiod probably checks the growth of some species it is not the only factor involved. Much more research will be necessary to give us a satisfactory understanding of the complex of factors controlling the course of growth and length of the growng season.

The writer wishes to acknowledge the cooperation of MR. J. S. HOLMES, State Forester of North Carolina and PROF. R. P. PRICHARD of the New York State College of Forestry in supplying tree seedlings, and the efficient assistance of MR. T. H. WETMORE in making the measurements.

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TRANSPIRATION RATES OF SOME FOREST TREE SPECIES DURING THE DORMANT SEASON

THEODORE T. KOZLOWSKI

(WITH TWO FIGURES)

This paper reports the results of a comparison of the winter transpiration rates of several deciduous and evergreen tree species. The species were: white oak (*Quercus alba* L.), yellow poplar (*Liriodendron tulipifera* L.), sugar maple (*Acer saccharum* Marsh.), cherry laurel (*Prunus laurocerasus* L.), eastern white pine (*Pinus strobus* L.), and loblolly pine (*Pinus taeda* L.).

Methods

Six individuals of each species, each about three years old, were transplanted from clay pots to cylindrical metal buckets of five-quart capacity. In transplanting the seedlings the entire core of soil containing the root system was transferred intact to reduce disturbance of the roots to a minimum. The soil used was a sandy loam with a field capacity of 23.2 per cent. After transplanting, the specimens were placed outdoors for a week prior to beginning experimental work. To prevent catchment of rain water and evaporation from the soil surface the buckets were sealed with two layers of oil cloth which sloped from the stems to the rims of the metal buckets at an angle of approximately 55 degrees. The oil cloth was heavily coated with a sealing compound consisting of paraffin, tallow, beeswax, and rosin. A glass tube, closed by a cork stopper, was inserted through each cover to permit the addition of water.

The experiment was performed on a flat roof where there was a minimum of shading by nearby trees and buildings. The specimens were arranged in six randomized blocks with each block having six plants representing each of the six species. The six blocks were located in a bed containing excelsior packing to prevent excessive changes in soil temperature as well as unnatural freezing. The blocks were so arranged that, although the afternoon shade affected the blocks differently, the plants within each block were shaded to the same extent. This arrangement was made to eliminate within-block variations due to shading differences.

The soil in the buckets at the beginning of each experimental period was at the previously determined field capacity, and the equivalent of the transpired water was replaced immediately after each experimental period. Weather data were obtained with a Friez hygrothermograph. Evaporation rates were obtained with standard Livingston atmometers. Total wind velocity for each experimental period was obtained with a cumulative recording anemometer. The gravimetric method of measuring transpiration was employed using a balance with a sensitivity of one gram. Loss in

weight during the experimental period was assumed to represent the weight of the water lost in transpiration.

The leaf areas of the broad-leaved evergreen species (*Prunus laurocerasus*) were determined by the photoelectric cell method modified and described by KRAMER (**6**). The stem surface areas of the deciduous species were obtained by taking diameter measurements at one inch intervals with a microcaliper along the stems and branches to establish the mean diameter. The diameter and total lengths of the stem and branches were readily converted to surface areas. The transpiring surface area (stomate bearing surfaces) of loblolly pine and eastern white pine were determined by the method described by KOZLOWSKI and SCHUMACHER (**5**). It was assumed that the transpiration rate from non-stomated leaf surfaces was negligible and these surfaces were disregarded in the calculations.

Results and discussion

Figure 1 indicates weekly losses for the evergreen species in grams of water transpired per square decimeter of stomate-bearing leaf surface and

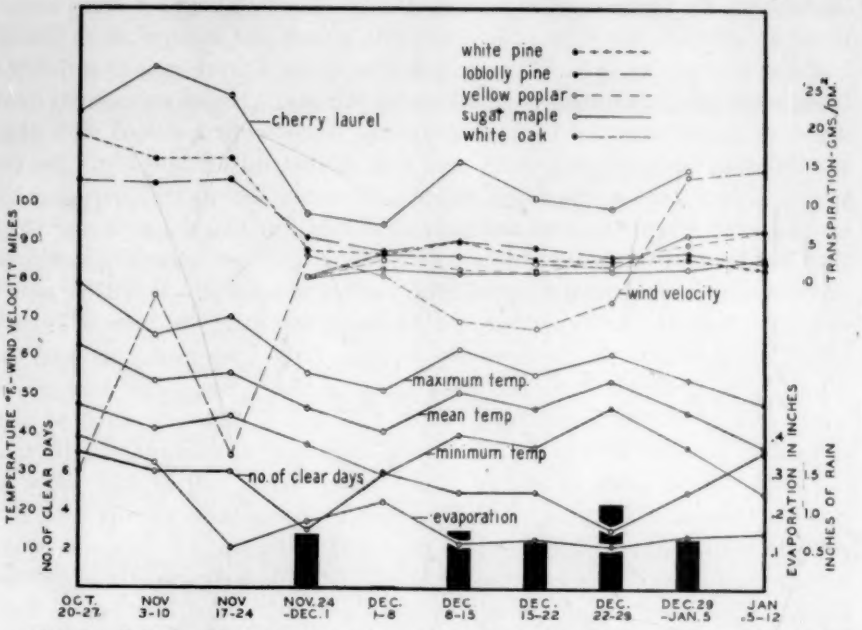

FIG. 1. Relationship between total weekly transpiration rates of six species during the dormant season and certain atmospheric variables. Data for six individuals of each species in Durham, North Carolina, from October, 1940, to January, 1941. Transpiration given in grams of water lost per week per square decimeter of transpiring area. Black vertical bars represent total rainfall in inches.

per square decimeter of exposed stem area for the deciduous species. Weekly losses for the evergreen species in grams of water transpired per gram of oven-dry weight of foliage are presented in table I. Data of figure 1 agree generally with the findings of WEAVER and MOGENSON (**10**) in that

they indicate no great differences between the transpiration rates of coniferous and deciduous species during the dormant season on a unit area basis. WEAVER and MOGENSON worked with broad-leaved and coniferous species under uniform conditions of soil type and texture, soil temperature, and identical aerial environment; they reported that autumn transpiration losses from conifers were just as great as, or even greater than, those from broad-leaved species. They found that winter losses by transpiration from conifers were only 1/55 to 1/251 as great as those in autumn. Increased losses of broad-leaved trees in spring occasioned by foliation were reported to be in proportion to the leaf areas exposed and were clearly controlled by weather conditions; although essentially, they were comparable to losses in conifers. They found that winter transpiration rates from conifers were little greater than those from defoliated stems of broad-leaved trees. The winter rates of these investigators were much lower than those reported herein, resulting in smaller seasonal differences than reported in their study.

Data of table I are in general harmony with findings of KUSANO (**8**) who presented quantitative data on transpiration of evergreen species indigenous to Japan. He found that in winter non-coniferous evergreen trees transpired an average quantity of at least 0.48 grams per square decimeter of leaf surface per day or 16.58 grams per 100 grams of fresh weight in foliage trees, while conifers transpired 8.18 grams per day. He also presented data which indicated that the time of minimum transpiration agreed with that of minimum temperature, which occurred at the end of January. He reported that on the average the amount of water lost in transpiration by broad-leaved evergreen trees was one and one-half or two times greater than that lost by conifers if the amount were reduced either to the fresh weight or to the dry weight of the transpiring part of the plant. It will be noted in table I that, during December and January, transpiration rates of cherry laurel when reduced to the oven-dry weight of the transpiring part of the plant were consistently higher than those of the pines. The amount of water lost by cherry laurel varied from 1.26 to 1.87 times as much as that of loblolly pine for weekly periods during December and January. During the same period, rates of cherry laurel varied from 1.80 to 3.11 times as great as those of white pine. During warmer seasons these relations did not seem to hold. Rates of both white pine and loblolly pine were somewhat higher than those of cherry laurel in late October on a dry weight of foliage basis. With lowering of average temperature, rates of cherry laurel were proportionally increased over those of the pines until the differences reported were recorded (table I).

Using transpiring surface area as a basis, the absolute rates of cherry laurel were consistently higher than those reported by KUSANO for broad-leaved evergreens. The average daily loss for cherry laurel was from 1.00 gram to 2.16 grams of water per square decimeter of leaf surface or the minimal loss was approximately twice that reported by KUSANO.

An analysis of variance as described by FISHER (**4**) was made for dor-

mant season rates on the basis of transpiring surface area from data for the six week period of November 24, 1940, to January 5, 1941; it was not until the former date that the deciduous species were completely defoliated. The analysis indicates that when all species are considered, highly significant effects on transpiration rates were due to species, time, and the interaction of time and species. The differences due to species are primarily due to the rates of cherry laurel which were higher than those of any other species when put on a leaf-area basis. The treatment or species sum of squares from the analysis was further broken up into five independent comparisons among the six species. A comparison between the evergreen species, including two pines and one broad-leaved species, against the deciduous species indicates significance at the one per cent. level. The indication is that

TABLE I

TRANSPIRATION OF EVERGREEN SPECIES IN GRAMS PER GRAM OF OVEN-DRY WEIGHT OF LEAVES FOR SEVEN-DAY PERIODS. DATA OF OCT. 20, 1940, TO JAN. 12, 1941. AVERAGE VALUES FOR SIX PLANTS OF EACH SPECIES

	LOBLOLLY PINE	WHITE PINE	CHERRY LAUREL
	gm.	*gm.*	*gm.*
Oct. 20 to 27	24.382	26.369	18.782
Nov. 3 to 10	23.983	23.520	23.497
Nov. 17 to 24	24.793	23.614	20.548
Nov. 24 to Dec. 1	10.348	7.324	7.363
Dec. 1 to 8	6.552	5.432	6.070
Dec. 8 to 15	9.643	7.312	13.136
Dec. 15 to 22	7.716	4.053	9.019
Dec. 22 to 29	5.523	3.440	8.029
Dec. 29 to Jan. 5	6.588	3.969	12.352
Jan. 5 to 12	3.846	1.783	.....

the observed differences would be due to chance not more than one time in one hundred. The magnitude of the real difference in rates between the pines and the cherry laurel against the deciduous species is indicated by a derived variance ratio of 46.68 whereas a ratio of only 7.77 would indicate significance at the one per cent. level for the degrees of freedom involved. The great difference between rates of the two pines and those of cherry laurel is indicated by a variance ratio of 56.21 for this independent comparison. The remaining independent comparisons were of white oak against yellow poplar, white oak against yellow poplar and maple, and yellow poplar against maple. These three comparisons were not statistically significant.

Soil temperature effects

The low winter transpiration rate of the conifers is partly caused by atmospheric factors reducing transpiration and partly by low soil temperature decreasing absorption. The importance of low soil temperature in decreasing transpiration was determined by growing plants in the greenhouse where the soil could be cooled while the air surrounding the tops was

maintained at normal greenhouse temperatures. A temperature control apparatus, consisting of four metal tanks, each equipped with a thermoregulator, heating coils, and a refrigerating unit was used. With this equipment the water in each tank could be maintained at any constant temperature from 0° to 40° C.

Seedlings were sealed in buckets, as in the experiments described above, and were randomized into four groups of 6 plants each. Three of the 6 plants in each group were loblolly pines and three were eastern white pines. Each water bath was adjusted to a constant temperature of 30° C. One group of buckets consisting of three white pines and three loblolly pines was placed in each tank so that each bucket was immersed. The position of the bucket within the tank was selected at random. The plants were kept in the water bath at 30° C. for three days in order to overcome the effects of the previous soil temperature. At the end of this preliminary three-day treatment each bucket was weighed and the moisture content of the soil within it brought back to the field capacity. Each plant was replaced in the position it had occupied within the tank and transpirational losses for a 90-hour period were determined gravimetrically. One tank was then maintained at 30° C. while the other three were adjusted to 0°, 5°, and 17° C. Three-day preliminary runs were made to overcome effects of previous soil temperatures before losses for 90-hour periods at the 4 temperatures were determined. After rerandomizing buckets between and within tanks, the entire second run was repeated. This yielded results essentially similar to those of the first run.

The transpiration rates of white pine and loblolly pine under the effects of varying soil temperatures are expressed as percentages of the rate at 30° C. in figure 2. Since one tank was maintained at 30° C. for both runs, it was assumed that, if atmospheric conditions were similar during both runs, transpiration rates of both runs would be of similar order, or the ratio in percentage would be 100. Since atmospheric conditions were not identical in successive runs some departure from the 100 per cent. ratio was recorded and a correction was necessarily applied to the rates of the plants in soils maintained at the lower temperatures.

In figure 2 it will be noted that for both species the maximum transpiration rate occurred at the highest control temperature, 30° C., and that a reduction in soil temperature reduced the rate of transpiration of both species. It is of interest to note the varying trends of the transpiration curves for these two species. For eastern white pine the average rate in a soil at 1° C. was approximately 21 per cent. of that of the control group; at 5° C. it was approximately 70 per cent. of that of the control group; and at 17° C. it was approximately 76 per cent. of that of the control group. For loblolly pine, however, the average rate at 1° C. was approximately 23 per cent. of that of the control group; at 5° C. it was approximately 51 per cent. of that of the control group; and at 17° C. it was approximately 74 per cent. of that of the control group. The results obtained are quite similar

to those of CLEMENTS and MARTIN (**2**) who investigated effects of soil temperature on the transpiration rate of *Helianthus annuus*, although they found a lesser reduction in transpiration from 30° to 15° C. They reported

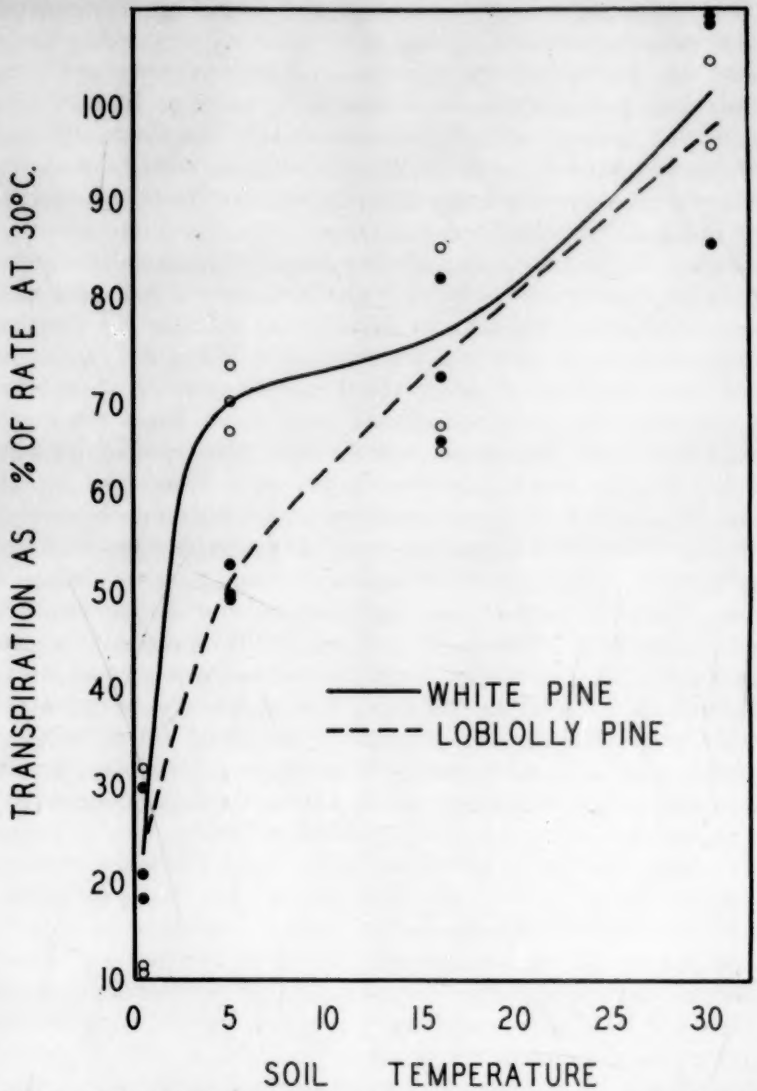

FIG. 2. Effects of soil temperature on transpiration rates of eastern white pine and loblolly pine. Observations for eastern white pine recorded as circles. Temperature in degrees Centigrade.

only a slight decrease in this range, but a very rapid decrease below 13° C., the reduction being one-half at 3° C. Plants began to wilt at about 4.5° C. and were completely wilted at 1° C. Warming the soil caused rapid recovery. ARNDT (**1**) reported that cotton in solution cultures wilted when their

temperature had been lowered to between 10° and 18° C.; in soil cultures, with ample water supply, the highest soil temperature at which wilting occurred was generally somewhat higher, between 17° and 20° C. DÖRING (3) used potometers and plants with roots submerged in water in studying effects of temperatures in the range of 20° to 0° C. on transpiration. He reported that decreases in transpiration rate due to lowered soil temperatures varied with species; the rate in some being decreased 70 to 80 per cent. while in other species it was not decreased at all. MICHAELIS (9) has suggested that high rates of transpiration when the soil is frozen and absorption is too slow to replace water loss may be an important factor in determining timber line in mountainous country.

Effects of low soil temperatures on water absorption and transpiration are operative in several ways which probably are interacting. KRAMER (7) cites several papers in summing up the following effects of low temperature on plant reactions: A decreased temperature decreases the rate of movement of water from the soil to the absorbing root surfaces. Low temperatures exert an effect in retarding root elongation. Since the continual extension of root tips into contact with the water films surrounding hitherto untouched soil particles is important in making soil moisture available a decrease or cessation of root elongation probably decreases the rate of absorption. This would be most important in soils with a moisture content below the field capacity, a condition which commonly exists in the field. Cell permeability is decreased as temperature is lowered while the viscosity of protoplasm and of colloidal gels in the cell walls is much higher at lower temperatures. The viscosity of water increases as temperature decreases and is twice as high at 0° as at 25° C. This results in a decreased rate of movement from soil to roots and through the root cells. Physiological activity, notably respiration rate, is markedly decreased by low temperatures.

Although absorption may be seriously affected by changes in the viscosity of the water itself, figure 2 indicates that colloidal properties of the protoplasm are also important in controlling absorption. The shape of the curve for loblolly pine more closely approaches that for the reciprocal of the viscosity of water than does the shape of the curve for eastern white pine. This suggests that low soil temperatures may cause a greater decrease in the permeability of the roots of loblolly pine to water than of those of eastern white pine. At low soil temperatures above freezing the greater reduction of absorption due to inherent protoplasmic qualities of loblolly pine might partly explain its inability to survive in colder regions.

Summary

An investigation was made of the absolute transpiration rates of loblolly pine, eastern white pine, cherry laurel, white oak, yellow poplar, and sugar maple during the dormant season of 1940–1941 in Durham, North Carolina. Absolute rates are reported for the 6 species in grams of water transpired per square decimeter of transpiring surface and also for the evergreen

species in grams of water transpired per gram of oven-dry weight of foliage. The effects of soil temperature on transpiration rates of loblolly pine and eastern white pine were studied.

The results agree generally with those of WEAVER and MOGENSON (**10**) in that no great difference was observed in foliar transpiration of conifers and stem transpiration of deciduous species on a unit area basis. Smaller differences between autumn and winter rates were found than those reported by these investigators. This was probably because their winter temperatures were much lower than those in the present experiments.

The October–November average rate for loblolly pine and cherry laurel was more than twice the average maximum for December and January; for white pine the October–November average rate was more than three times the December–January maximum.

Independent comparisons of the evergreen versus deciduous species and of pines versus cherry laurel indicated highly significant differences on a unit area basis. During December and January the weekly transpiration rates of cherry laurel were approximately from 2 to 4 times as great as those of either of the pines. Comparisons of rates of loblolly pine versus eastern white pine, white oak versus yellow poplar and sugar maple, and yellow poplar versus sugar maple indicated no real statistical differences in transpiration of these species during the dormant season.

A decrease in soil temperature was found to decrease the transpiration rates of loblolly pine and eastern white pine. This is in general agreement with results obtained by other investigations with herbaceous materials. Transpiration of loblolly pine was reduced more than that of eastern white pine over the temperature range between 17° and 0° C. The difference in behavior of the two species is probably caused by differences in inherent protoplasmic qualities. The greater reduction in absorption by loblolly pine in cold soil might be a factor in its inability to survive in colder regions.

The writer is indebted to DR. PAUL J. KRAMER for kindly counsel throughout this investigation and for critical review of the manuscript. Indebtedness is also acknowledged to PROFESSOR F. X. SCHUMACHER for advice on experimental design and data analysis.

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A NOTE ON THE GROWTH BEHAVIOR OF COTTON BOLLS[1]

DONALD B. ANDERSON AND THOMAS KERR

(WITH SEVEN FIGURES)

The wall of every cotton fiber is made up of two component parts: (a) the primary wall and (b) the secondary wall (1). The primary wall, an extremely thin layer deposited by the elongating epidermal cell, is formed during a period of about sixteen days following the opening of the cotton flower. During this period the fiber reaches its full length, but the wall remains extremely thin and delicate. About the time that the fiber ceases to elongate the second phase of its development, that of wall thickening, begins. Wall thickening occurs continuously and simultaneously over the entire inner surface of the fiber for the succeeding twenty or more days. The wall deposited during this second phase of development is the secondary wall of the fiber cell. It is this secondary wall that is responsible for the tensile strength of the cotton fiber.

The tensile strength of cotton fiber is influenced by many factors, both environmental and genetic. In the course of an investigation of these factors, it was noticed that the tensile strength of the fibers appeared to increase whenever the plants were subjected to a deficiency of water during the period of secondary wall formation. This observation suggested that measurements of water stress within the plant at the time of secondary wall deposition might serve as an index to the tensile strength of the mature fiber. Efforts were made therefore to obtain some record of the variations in water stress within the plant during the period of fiber growth.

The most obvious indication of the severity of water stress in plant cells is the magnitude of cell turgor. Variations in the turgor of the cells composing the young seeds and bolls should be reflected by corresponding changes in the volume and in the diffusion pressure deficits of the cells of these organs. Measurements of these quantities were undertaken and this paper is a report of the results of studies of the changes in the volume of growing cotton bolls during the summers of 1940, '41, and '42.

Methods

Continuous records of the diurnal and seasonal changes in the diameters of cotton bolls were obtained with auxometers designed for this purpose. A young cotton boll was pressed lightly against a vertical glass plate by a movable lever arm in such a way that any change in the diameter of the boll would induce a movement of the lever arm. The slight movements of the lever arm were multiplied by a series of levers so that any changes in

[1] Cooperative investigations between the Division of Cotton and other Fiber Crops, Bureau of Plant Industry and the North Carolina Agricultural Experiment Station. Approved for publication as Paper no. 151 of the Journal Series of the North Carolina Agricultural Experiment Station.

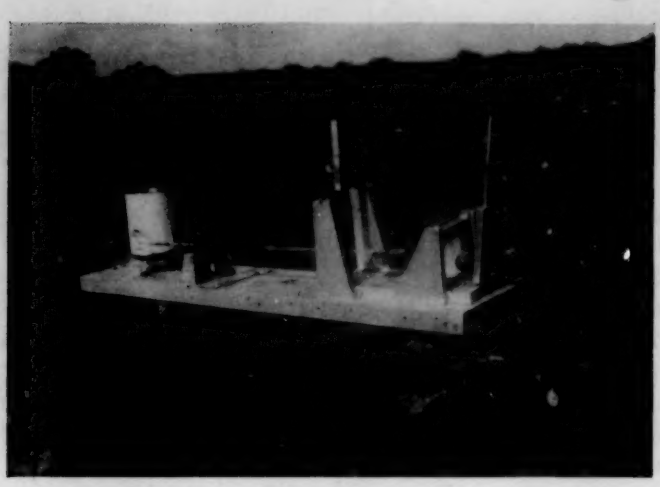

FIG. 1. The auxometer. A detached boll has been placed between the lever arm and the glass plate—(right end of the apparatus)—to illustrate the position that the boll occupies when the machine is in operation.

boll diameter were increased about 40 times at the point where they were recorded, in ink, upon a rotating drum. The apparatus (fig. 1) was constructed largely of wood with a pen arm of glass tubing supported upon bronze knife-edge bearings by a short brass axle. The wood was protected from expansion or contraction resulting from the absorption or loss of water by means of aluminum foil. Each piece of wood in the instrument was covered, before assembly, with five layers of aluminum foil cemented together with white lead and linseed oil (**5**). This method of protecting the

FIG. 2. The auxometer mounted in the field. The rotating drum is covered with a metal housing with a glass window to permit observation. The boll is shaded from the direct sunlight by means of a small square of plywood which is visible at the right.

wood was entirely successful so that in a test run, with a quartz pebble substituted for a cotton boll, a straight line was recorded on the drum during a very warm day followed by a severe thunder storm. The rotating drums used were those manufactured by Julien P. Friez and Sons for their recording thermometers. The pen recording mechanism was similar to that employed on the Friez instrument just mentioned. Records were obtained every day upon thermograph charts, the change of charts being made about sunset in 1940–1941, and at 8:30 A.M. (war time) in 1942.

The auxometer was mounted upon a 3-ft. length of a $2'' \times 6''$ plank which in turn was supported upon stakes driven solidly into the ground (fig. 2). The branch bearing the boll was tied firmly in place with raffia, and the main stem of the plant was likewise securely fastened with raffia to a substantial stake. The bolls were held in place so firmly that the position of the boll in the instrument was not altered by winds prevalent during the summer.

Since it was important for our purpose to determine the diurnal and seasonal variations in the diameter of bolls growing under field conditions, the instruments were mounted in fields of the North Carolina Agricultural Experiment Station Farm at Raleigh. Five auxometers were operated simultaneously in a single experimental plot so that records were obtained of the growth behavior of bolls of different ages under the same environmental conditions. Although measurements of diameter changes in bolls of several strains of upland cotton (Rowden, Half and Half, Coker 100, Cook, and D & P L) were obtained during the three summers' work no significant differences in behavior were observed in any of the varieties studied.

It is recognized that changes in the *diameter* of a globose fruit like the cotton boll do not represent very adequately the actual magnitude of the variations in *volume* since the relation of the volume of a sphere to the radius is expressed by the formula $4/3\,\pi R^3$. The total volume changes in the boll are, of course, much greater than those indicated by the growth curves presented in this paper. The records of the diameter changes as recorded by the auxometer do indicate, however, the general pattern of growth behavior very well, and it has seemed better for various reasons to use the auxometer records directly rather than to convert these into volume changes. All of the growth curves presented in this paper are, therefore, variations in the diameter of the bolls as recorded automatically by the instrument.

Results

It soon became apparent that the response of young, enlarging bolls to the internal water stress of the parent plant was entirely unlike that of full-sized bolls. So long as the boll was growing in size, *i.e.*, during the period of primary wall formation in the fiber cells, the increase in diameter was continuous during both the day and the night. Enlargement during the day exceeded that which occurred during the night, indicating that the higher temperatures of the day had a greater influence upon growth than the more favorable water relations existing during the night. This rapid enlargement

of the young bolls (7 to 12 days old) during the day continued to occur during periods of severe drought when the leaves of the cotton plants were usually wilted by 10 A.M. and even when the leaf blades were hanging limply from the petioles in the afternoon (fig. 3). As the bolls approached full size (13 to 15 days), however, the rate of enlargement was noticeably checked by severe wilting of the parent plant and sometimes slight shrinkage of the bolls occurred.

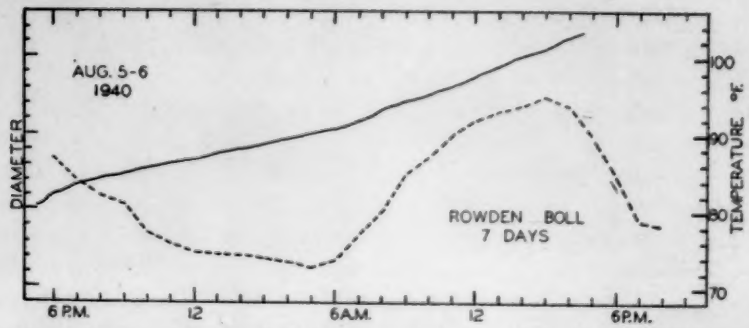

FIG. 3. Diurnal variations in the diameter of a young cotton boll during a 24-hour period in August (solid line). Temperature variations are shown by the broken line. The parent plant exhibited marked wilting before noon but the water stress within the plant failed to check enlargement.

As soon as the bolls reached their full size, *i.e.*, as soon as the secondary walls began to form in the fibers, the pattern of diurnal variations in diameter was completely altered. The bolls decreased in size during the warm part of the day when wilting of the parent plant occurred and regained their full size during the night (fig. 4). When soil moisture was near field

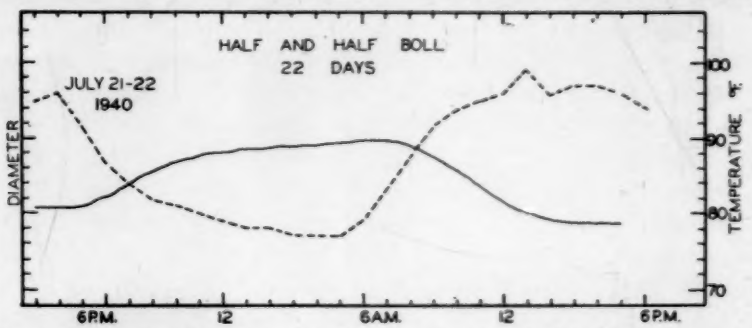

FIG. 4. Diurnal variations in the diameter of a full-sized boll during a warm day and night in July (solid line). Temperature variations are shown with the broken line. Shrinkage begins in the morning about the time that wilting is visible in the leaves.

capacity some shrinkage in size of the bolls occurred before the first evidence of wilting became visible in the leaves. As soil moisture decreased, shrinkage increased in amount during the day and recovery was slower during the night. Under these conditions shrinkage did not occur in the bolls until after the leaves were visibly wilted. The amount of shrinkage was in general directly proportional to the degree of the wilting visible in the leaves.

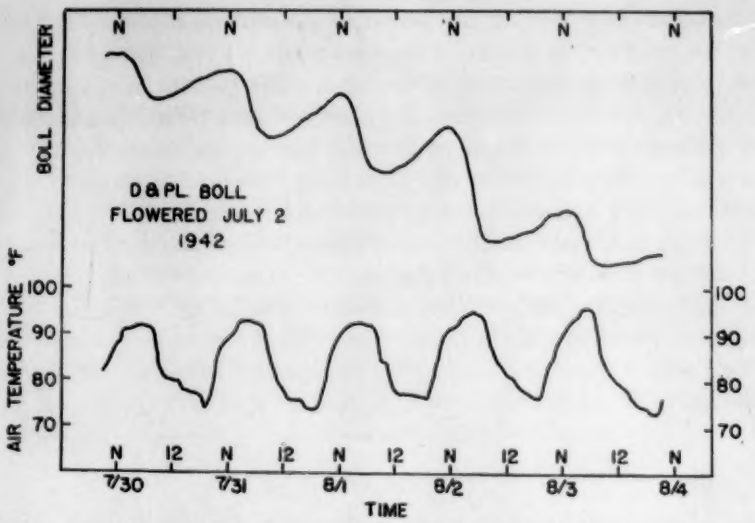

FIG. 5. Variations in the diameter of a full-sized boll during a period of severe drought. The plant was approaching permanent wilting on Aug. 4.

In the summer of 1942, drought was so severe that the cotton plants closely approached permanent wilting. Under such severe water stress, the full sized bolls continued to show shrinkage during the day but the decrease in diameter did not begin until early in the afternoon although the leaves were severely wilted by 10 A.M. They did not recover completely at night so that the bolls became smaller day after day (fig. 5). When permanent wilting occurred there was practically no recovery of bolls during the night.

In figure 6 the growth behavior of a full sized boll is shown in the six

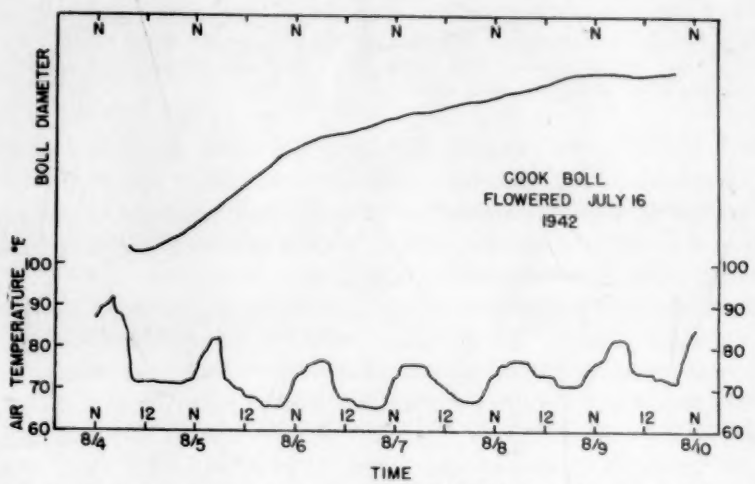

FIG. 6. Variations in the diameter of a full-sized boll as it recovered from severe drought.

days immediately following the period of permanent wilting. During this six-day interval the air temperatures were relatively low, rains were frequent and the sky was overcast most of the time. The boll increased in size continuously but not uniformly until the plant attained its maximum turgor by which time the boll exhibited no further increase in diameter. A similar pattern of growth behavior was shown by the boll in each of the five auxometers. When the plants are turgid an hour or two of sunshine in the middle of the day is sufficient to induce some shrinkage in the full sized bolls. This behavior stands in marked contrast to the severe wilting that is necessary to induce shrinkage when soil moisture is seriously deficient.

Figure 7 represents a continuous record of the growth of a single cotton boll for a period starting 11 days after the opening of the flower and ending 16 days later. The marked contrast between the diurnal variations in volume

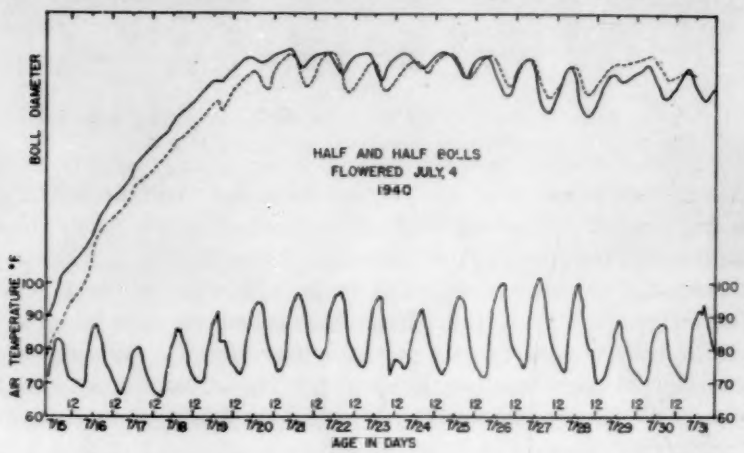

FIG. 7. Variations in the diameters of two cotton bolls starting 11 days after flowering and ending 16 days later. When young, the bolls do not shrink during the warm part of the day but as soon as they attain full size a marked shrinkage occurs in the middle of the day. Rain prevented shrinkage on July 29.

of the boll during its enlargement phase and those that occur after the cells have attained their mature dimensions is strikingly apparent. During enlargement the higher temperatures of midday increased the rate of growth but as soon as the cells reached their maximum size the higher temperatures of midday resulted in conspicuous shrinkage.

Severe drought decreases the daily increments of growth without changing its general pattern. Young bolls continue to enlarge more rapidly during the day, even though the plants are severely wilted, than at night when moisture conditions are more favorable. The total amount of each day's increase in size is less, however, than that which occurs in bolls that undergo their enlargement when the water supply is more adequate. The duration of the period of enlargement is also less in very dry weather (15 days) than when water is not a limiting factor (16 to 18 days). The effect of dry

weather in decreasing both the daily increments of growth and the period during which increase in size occurs, is to reduce the size of the mature bolls. Mature bolls which underwent the enlargement phase of their growth during the exceptionally dry weather of late July, 1942, were only about half the size of bolls formed in the same plants earlier and later in the season when the plants were not subject to severe wilting.

Boll enlargement and fiber elongation are parallel processes that terminate at about the same time. Shortening the period of boll enlargement should, therefore, shorten the cotton fibers. STURKIE (**11**) has shown previously that fibers developed during periods of water stress are distinctly shorter than fibers formed when the period of enlargement has not been curtailed by deficient water. Insofar as the effect of soil water upon fruit size is concerned, cotton seems similar in its response to lemons (**2**), apples (**10**), peaches (**6**), pears (**4**) and many other fruits.

Discussion

Shrinkage in the size of vegetative and reproductive organs is a very common result of deficient water. Often as in the case of the full sized cotton bolls discussed in this paper the shrinkage is merely a temporary consequence of rapid transpiration, low water absorption or both. The diurnal variations in the diameter of lemon fruits reported by BARTHOLOMEW (**2**) and those found by MACDOUGAL (**8, 9**) to occur in various fruits and even in good sized tree trunks seem essentially similar to the variations here reported for full sized cotton bolls. The shrinkage observed in full sized cotton bolls at the time of wilting of the parent plant is undoubtedly the result of a movement of water out of the boll to the leaves and other organs where the diffusion pressure deficits of the cells exceed those of the boll. Such an internal redistribution of water is a very common phenomenon in plants and is thoroughly familiar to all plant physiologists.

The continued enlargement of the young bolls during periods in which the parent plant exhibited severe wilting was not anticipated and seems contrary to the general experience of plant physiologists. It has long been recognized that the exposure of plants to full sunlight inhibited elongation and the fact has commonly been ascribed to a reduction in the turgor of the cells in the region of enlargement [LOOMIS, (**7**)]. In cotton, however, water apparently continues to enter the young bolls when it is being lost by full sized bolls, leaves and other vegetative organs. MACDOUGAL (**8**) has reported that young growing cells of sunflower stems continued to increase in size when cells in mature regions were shrinking as a result of deficient water. This fact, as MACDOUGAL points out, makes it necessary to consider carefully the cellular organization of any tissue before attempting to evaluate the effect of deficient water upon tissue enlargement. The shrinkage of a stem at midday does not necessarily indicate a stoppage of cell enlargement. Young cells in the stem may continue their enlargement but the increase in size may be completely masked by the decrease in size of the mature cells. The number of mature cells so greatly exceeds the

number of enlarging cells that the net results of deficient water will be a decrease in size even though enlargement of young cells continues uninterruptedly. In cotton bolls between the date of flowering and about 16 days later, the cells of the carpel wall and of the immature seeds continue to enlarge and hence to receive water at the expense of more mature cells. As soon as the bolls reach an age of 16 days the cells have reached their mature dimensions and no longer seem able to maintain their turgor when the plant is subjected to stress conditions.

The explanation of the high water absorbing capacity of the enlarging bolls cannot be considered in detail in this report but it does not seem to be the result of any simple osmotic mechanism. The cells of immature cotton seeds and carpel wall do not appear to have higher diffusion pressure deficits than the more mature cells of bolls that have completed their enlargement. The osmotic pressures and diffusion pressure deficits of enlarging and full sized cotton bolls will be discussed in a subsequent paper.

The importance that the high water absorbing capacity of enlarging bolls may have in affecting the water relations of the plant were strikingly revealed during the extremely dry weather of late July and early August 1942. Cotton plants of the same variety, but differing in age by about two weeks, were growing in adjacent rows in the field. The older plants had relatively few enlarging bolls while most of the bolls on the younger plants were in the enlargement phase of growth. The leaves of the younger plants wilted earlier than those of the older plants and by midday the greater severity of the wilting of the younger plants was conspicuous. In the older plants the bolls served as reservoirs from which water moved into the wilting leaves but in the younger plants the enlarging bolls continued to extract water from the vegetative tissues thus accentuating the low turgor produced by the high transpiration rate.

Summary

1. Continuous records of the diurnal and seasonal variations in the diameters of cotton bolls of different varieties have been recorded during the summers of 1940, 1941, and 1942.

2. The enlargement of young bolls is not inhibited by severe wilting of the parent plant.

3. Full sized bolls shrink in size when the parent plants are visibly wilted and regain their size during the night if low soil moisture is not a limiting factor.

4. The degree of shrinkage of full sized bolls is, in general, proportional to the severity of wilting of the parent plant.

5. During periods of severe drought shrinkage occurs later in the day and recovery during the night is only partial or even entirely absent.

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POLLEN GERMINATION AND POLLEN TUBE GROWTH, AS INFLUENCED BY PURE GROWTH SUBSTANCES[1]

FREDRICK T. ADDICOTT

Investigators studying the germination of pollen and growth of pollen tubes have, in the past, of necessity concerned themselves with restricted aspects of the problem. Most of the reports now in the literature deal with pollen germination as it has affected some aspect of cytology, genetics, or plant breeding. As a result, our knowledge of the physiology of pollen tube growth is far from complete; the need for a thorough understanding of the subject, however, is readily recognized. Recently a few reports of work with certain growth promoting substances on pollen germination have appeared (**1, 3, 6**). This paper extends previous work, and presents the results of experiments on germination of pollen and the growth of pollen tubes in response to 33 pure growth substances and several impure preparations. To the investigator's knowledge this work is the first in which any of the substances have been tested in the presence of optimum concentrations of boron, now known to be essential to the germination of the pollen of many species.

The germination of pollen and growth of the pollen tube are complex phenomena involving several morphological and physiological processes. Morphologically there is in germination a swelling of the pollen grain followed by protrusion of the tube. The tube nucleus usually remains in the cytoplasm near the tip of the tube. The generative cell may enter the tube from a few minutes to many hours after the appearance of the tube, depending on the species. Its division into two sperm nuclei usually takes place after entrance into the tube. *In vivo* the tube grows from the stigma to the vicinity of a female gametophyte and bursts, liberating its three nuclei. The distance it grows may be from a few millimeters to nearly one hundred. *In vitro* germination may approximate that on the stigma, but it is doubtful if normal tube growth has yet been obtained. Tubes often show various abnormalities. They may be short, granular, vesicular, thin, bifurcated, or various combinations of these.

Physiologically, the pollen tube requires several types of nutrients: (a) it requires water; (b) it requires inorganic salts; (c) it requires a source of energy (usually supplied as sugar); (d) it requires a complement of growth factors of hormonal or vitamin nature. Pollen may, however, supply many of these requirements from its own reserves. There is apparently considerable variation between species in this regard. Some pollen can germinate in distilled water (**1**). Other types, such as the pollen of Milla and Tropaeolum used in this investigation, apparently require nutrients from all four of the above categories.

[1] Report of an investigation carried out with the assistance of a Grant-in-Aid from the Society of Sigma Xi.

Materials and methods

The pollen of two species was selected for the experiments: *Milla biflora*, a monocotyledon; and *Tropaeolum majus*, variety Golden Gleam, a dicotyledon. These were chosen because they represented the two principal groups of flowering plants, and their flowers could be obtained throughout the period available for experimentation.

A number of methods were tested for growing pollen tubes *in vitro*. Those involving agar or gelatin did not permit as ready observation during the period of growth as did the hanging drop method. The method involving hanging drops on the under side of the cover of a Petri dish was finally selected. Eight drops of the medium to be tested were placed on the cover and about 1 ml. of distilled water in the bottom of the dish, to maintain a saturated atmosphere. The drops had an average volume of

TABLE I

EFFECT OF THE COMPONENTS OF THE BASIC MEDIUM ON GERMINATION AND GROWTH OF THE POLLEN TUBE IN MILLA

MEDIUM	PERCENTAGE GERMINATION*	POLLEN TUBE LENGTH*
	%	%
Distilled H_2O	3	42
Distilled H_2O + 0.01% H_3BO_3	0	0
Distilled H_2O + 12% glucose	3	42
HOAGLAND and ARNON'S sol'n (less boron)	0	0
" " " " + 0.01% H_3BO_3	8	42
" " " " + 12% glucose	45	47
" " " " + 0.01% H_3BO_3 + 12% glucose	100	100

* These values are expressed in terms of the controls taken as 100 per cent. In this series the actual value of the controls were: germination 62 per cent., tube length 2.3 diameters of the pollen grain. Each value is based on 60 to 120 pollen grains.

0.005 ml. which was obtained through the use of a fine pipette. Pollen from Milla was placed in four of the drops and pollen from Tropaeolum in the other four.

From 15 to 30 pollen grains were placed in each drop. In the case of Milla, pollen was taken from flowers which had opened within the past 12 hours. In the case of Tropaeolum, whose anthers open much later than the petals, pollen was taken from anthers which had opened in the past 12 hours. It was found that the viability of the pollen decreased with age. The size of the drop and the amount of inoculum were kept as constant as possible. It was noted that pollen tube growth was greatly increased when the number of grains was high in proportion to the amount of medium present. Since this phenomenon was presumably due to a diffusible chemical, the inoculum was kept low so that the chemical would be detected if it were among those added to the medium.

The basic medium contained water double distilled from Pyrex glass, HOAGLAND and ARNON's solution I (supplying all the known inorganic

nutrients of plants) in which the boric acid content had been raised to 0.01 per cent. (5), and 12 per cent. glucose. The latter two concentrations were found to be optimum for both Milla and Tropaeolum. To this basic medium were added various pure growth factors and impure preparations in at least five different concentrations. The boric acid requirement is in accordance with the findings of SMUCKER (7), COOPER (3), and WEBB (unpublished data). Five or more concentrations of glucose, sucrose, fructose, and glycerol were tested as sources of carbohydrate. Optimum germination and tube growth were obtained with 12 per cent. glucose in both species.

In recording results the percentage of germination and the length of the tubes were taken two hours after inoculation. Growth always ceased

TABLE II

EFFECTS OF VARIOUS IMPURE PREPARATIONS ON GERMINATION AND GROWTH OF THE POLLEN TUBE IN TROPAEOLUM AND MILLA

PREPARATION ADDED TO BASIC MEDIUM	CONCENTRATION	PERCENTAGE GERMINATION*		POLLEN TUBE LENGTH*	
		TROPAEOLUM	MILLA	TROPAEOLUM	MILLA
		%	%	%	%
Pollen	To cover the surface of the drops	133	141	156	181
Intact stigma	For Milla ½ stigma per drop was used	91	102	270	112
Minced stigma		91	92	65	79
Minced style		95	34	43	74
Stigma exudate of Milla	About 10%	131	104	186	163
Stigma exudate of Milla	100%	—	120	—	850
Beef extract (Difco)	1 mg. per liter	97	95	73	89
Peptone (Difco)	1 mg. per liter	96	94	167	99
Spanish saffron	80 mg. extracted with a liter of basic medium	149	140	183	115

* These values are expressed in terms of the corresponding controls which were taken as 100 per cent. in each case. Each value is based on 60 to 120 pollen grains.

before this time had elapsed. As no constant temperature apparatus was available the cultures were kept at room temperature. This was always close to 25° C. and never at any time during the experimental periods did the temperature vary more than 2° from 25° C. The length of the tubes was measured in terms of pollen grain diameters. This was a convenient unit which was quite uniform. The pollen grains of Milla have more than twice the diameter of those of Tropaeolum. The figures in the tables are based on averages from each experiment employing from 50 to over 100 pollen grains. The data in the tables have been expressed in terms of percentages of the amount of germination or growth in the "control" of each experiment. The "control," or basic medium, consisted of the salt solution and sugar mentioned above.

Observations

The effects of various factors in the basic medium are shown in table I. The value of glucose and a high concentration of boric acid is apparent.

Table II shows the effects of impure preparations and extracts when added to the basic medium. The observations of VASIL'EV (8), GOTOH (4) and others that the stigma and its exudate are active in promoting the growth of the pollen tube are demonstrated. That this growth, however, is not due entirely to the boron content of the stigma is apparent from the

TABLE III

EFFECTS OF PURE GROWTH FACTORS ON GERMINATION AND TUBE GROWTH OF TROPAEOLUM POLLEN

GROWTH SUBSTANCE	GROWTH SUBSTANCE CONCENTRATION MILLIGRAMS PER LITER									
	100		10		1.0		0.1		0.01	
	GERMINATION	TUBE LENGTH	GERMINATION	TUBE LENGTH	GERMINATION	TUBE LENGTH	GERMINATION	TUBE LENGTH	GERMINATION	TUBE LENGTH
	%	%	%	%	%	%	%	%	%	%
Thiamin	95	66	90	244*	90	104	112	111	93	85
Niacin	53	45	80	90	94	104	77	110	81	110
Niacinamide	80	114	96	164	79	126	89	95	93	74
Riboflavin	98	145	94	100	90	76	98	91	95	112
Para-aminobenzoic acid	48	33	94	96	84	84	94	84	97	100
Inositol	95	79	99	84	93	91	95	70	108	164
Pyridoxin	97	80	99	68	94	77	54	74	77	77
Ascorbic acid	103	41	92	115	94	81	96	69	92	124
Alpha-naphthyl acetamide	38	99	85	89	53	109	106	213*	109	64
Indoleacetic acid	4	33	92	91	91	114	78	62	96	137
Traumatic acid	14	31	54	31	101	77	109	65	70	49
Uric acid	114	85	117	139	88	126	56	139	97	107
Adenine	90	116	103	125	100	107	98	86	103	83
Thymine	64	83	66	45	107	49	79	114	67	91
Cytosine	117	168	117	101	93	123	130	76	78	127
Uracil	103	132	56	94	84	89	88	92	103	89
2-methyl-4,6-dihydroxy-purine	112	67	95	54	83	81	85	51	87	81
2-methyl-4-hydroxy-5-hydroxymethylpurine	68	159	71	113	100	68	86	83	74	117
2-methyl-4-amino-5-thio-formamidomethylpurine	30	117	34	136	92	53	95	99	119	76
2-methyl-4-hydroxy-5-aminomethylpurine-hydrochloride	0	0	74	79	112	87	64	121	79	114
2-methyl-4-amino-5-amino-methylpurine-hydro-chloride	93	125	77	79	67	110	73	136	86	106
Pimelic acid	3	49	74	49	129	59	91	64	96	79
Allantoin	78	92	35	58	90	86	89	92	89	83
Alloxan	79	95	74	80	102	95	80	125	90	92
2-chloroisothiamin-iodide	93	92	100	75	121	97	55	97	113	78

* Significant deviations from the controls.

fact that the medium already contained the optimum concentration of boric acid. Since it also contained the full complement of inorganic chemicals known to be essential to plant growth it seems highly probable that the effects of the stigma are due at least in part to one or more of its organic constituents. This suggestion is supported by the fact that an extract of saffron also shows some activity.

Germination and pollen tube growth were also promoted by crowding the pollen. This appears to be parallel to the well known "bios effect" in yeast and other microorganisms where the growth of organisms is stimulated when the inoculum contains large numbers. This effect in pollen has

TABLE IV

EFFECTS OF PURE GROWTH FACTORS ON GERMINATION AND TUBE GROWTH OF MILLA POLLEN

GROWTH SUBSTANCE	GROWTH SUBSTANCE CONCENTRATION MILLIGRAMS PER LITER									
	100		10		1.0		0.1		0.01	
	GERMINATION	TUBE LENGTH	GERMINATION	TUBE LENGTH	GERMINATION	TUBE LENGTH	GERMINATION	TUBE LENGTH	GERMINATION	TUBE LENGTH
	%	%	%	%	%	%	%	%	%	%
Thiamin	75	51	87	59	77	67	72	57	94	53
Niacin	96	90	92	71	53	127	85	80	86	68
Niacinamide	86	56	97	49	110	34	88	74	64	102
Riboflavin	118	142	87	112	95	107	107	208*	112	142
Para-aminobenzoic acid	164*	80	78	214*	203*	117	193*	104	163*	110
Inositol	176*	153	190*	123	161*	110	180*	153	149	141
Pyridoxin	109	72	106	105	88	83	43	139	78	244*
Ascorbic acid	92	98	105	84	100	80	102	110	94	87
Alpha-naphthyl acetamide	23	110	62	146	105	110	115	139	103	160
Indoleacetic acid	67	61	82	142	74	178	74	244*	82	107
Traumatic acid	0	0	13	55	62	117	100	133	84	117
Uric acid	67	31	90	41	97	63	51	20	67	57
Adenine	106	94	108	76	109	50	110	68	112	60
Thymine	102	128	102	94	93	168	94	188*	102	87
Cytosine	76	51	89	98	96	104	100	76	97	109
Uracil	137	107	142	139	153*	79	108	79	137	170
2-methyl-4,6-dihydroxypurine	140	99	127	123	142	103	130	95	149	91
2-methyl-4-hydroxy-5-hydroxymethylpurine	98	168	97	114	98	141	98	101	98	128
2-methyl-4-amino-5-thioformamidomethylpurine	102	154	98	228*	95	114	96	248*	99	168
2-methyl-4-hydroxy-5-aminomethylpurine-hydroehloride	99	262*	100	141	99	74	95	188*	98	101
2-methyl-4-amino-5-aminomethylpurine-hydrochloride	99	202*	99	141	102	322*	99	154	99	101
Pimelic acid	106	110	112	110	114	102	108	110	108	110
Allantoin	110	57	99	102	105	86	105	78	112	65
Alloxan	103	39	104	44	108	52	112	60	104	70
2-chloroisothiamin-iodide	0	0	11	71	19	191*	124	147	148	167
Ca-pantothenate	110	130	84	99	73	57	124	205*	126	213*

* Significant deviations from the controls.

also been recently noted by BECK and JOLY (1). With both species no tropistic growth was observed either towards or away from clumps of pollen. The direction of growth appeared to be completely random, regardless of the arrangement of the pollen.

TABLE V

EFFECTS OF PURE GROWTH FACTORS ON GERMINATION AND TUBE GROWTH OF TROPAEOLUM POLLEN

GROWTH SUBSTANCE	GROWTH SUBSTANCE CONCENTRATION (IN TERMS OF SATURATION)									
	SAT'D		0.1 SAT'D		0.01 SAT'D		0.001 SAT'D		0.0001 SAT'D	
	GERMINATION	TUBE LENGTH	GERMINATION	TUBE LENGTH	GERMINATION	TUBE LENGTH	GERMINATION	TUBE LENGTH	GERMINATION	TUBE LENGTH
	%	%	%	%	%	%	%	%	%	%
Alpha-tocopherol	98	56	113	96	85	81	91	107	108	107
Estrone	129	130	110	92	129	115	95	121	126	143
Xanthine	98	141	133	94	64	89	46	98	92	120
Guanine	129	83	100	76	116	101	115	55	128	82
4-methyl-5-hydroxymethyl-uracil	124	152	119	74	77	117	42	130	100	74

Tables III to VI present the results of tests of pure substances. The starred (*) values represent significant deviations from the controls. Significant differences were found to be an increase in germination of 51 per cent. and an increase in tube length of 85 per cent. over the controls. The terms "significant difference" as here used indicates that analysis showed that there was less than 1 possibility in 20 that the observed difference was due to pure chance. Sixteen of the substances tested affected germination or tube growth when judged on this basis.

The above results were all from tests with single substances. Table VII shows the results of experiments with two mixtures of pure substances.

TABLE VI

EFFECTS OF PURE GROWTH FACTORS ON GERMINATION AND TUBE GROWTH OF MILLA POLLEN

GROWTH SUBSTANCE	GROWTH SUBSTANCE CONCENTRATION (IN TERMS OF SATURATION)									
	SAT'D		0.1 SAT'D		0.01 SAT'D		0.001 SAT'D		0.0001 SAT'D	
	GERMINATION	TUBE LENGTH	GERMINATION	TUBE LENGTH	GERMINATION	TUBE LENGTH	GERMINATION	TUBE LENGTH	GERMINATION	TUBE LENGTH
	%	%	%	%	%	%	%	%	%	%
2-methyl-1,4-naphthoquinone	8	46	5	46	92	109	142	114	129	180
Alpha-tocopherol	98	66	93	103	106	79	106	74	91	79
Estrone	84	60	85	120	108	74	95	86	98	55
Xanthine	65	37	93	101	112	90	108	103	107	94
Guanine	82	27	105	140	127	203*	141	257*	129	138
4-methyl-5-hydroxymethyluracil	86	81	97	89	102	140	90	144	102	127
Acenaphthene	126	154	147	197*	139	199*	63	90	153*	130

* Significant deviations from the controls.

These combinations did not appreciably add to germination or tube growth over that of single substances.

Table VIII presents a classification of all the pure substances tested and indicates which were effective in promoting germination or pollen tube growth.

TABLE VII

EFFECTS OF MIXTURES OF PURE SUBSTANCES ON GERMINATION AND GROWTH OF POLLEN TUBES

CONCENTRATION	SUBSTANCE	PERCENTAGE GERMINATION		TUBE LENGTH	
		TROPAEOLUM	MILLA	TROPAEOLUM	MILLA
1 mg./l.	Indoleacetic acid	150	112	80	147
10 mg./l.	Niacinamide				
10 mg./l.	Thiamin				
100 mg./l.	Riboflavin				
0.01 mg./l.	Inositol				
0.1 sat'd	Estrone				
0.01 sat'd	Guanine				
0.1 mg./l.	Indoleacetic acid	—	43	—	147
0.1 mg./l.	Niacin				
0.1 mg./l.	Thiamin				
0.1 mg./l.	Riboflavin				
0.1 mg./l.	Inositol				
0.1 mg./l.	Pyridoxin				
0.1 mg./l.	Ascorbic acid				
0.1 mg./l.	Para-aminobenzoic acid				
0.1 mg./l.	Ca-pantothenate				
2%	Gum Tragacanth				
To give pH 4.4	Citric acid				

Discussion

From these experiments it appears that germination of the pollen grain and the subsequent growth of the pollen tube are not necessarily related phenomena. They can be stimulated independently. For example, inositol increased the germination of Milla pollen up to 90 per cent. over that of the controls without greatly affecting the length of the tubes. Guanine, on the other hand, increased the length of the pollen tubes of Milla up to 157 per cent. more than that of the controls, without significantly affecting the percentage of germination. Two substances, para-amino-benzoic acid, and acenaphthene affected both processes.

It is of interest to note that several classes of substances show activity in the germination and growth of pollen. These include vitamins, plant hormones, and pyrimidines and purines. The latter group of substances should perhaps be classified with the plant hormones since the purines are active as leaf growth factors. They were kept separate, however, for convenience in handling a large group of chemically distinct compounds. Several vitamins are known to be growth hormones in the higher plants or growth factors for the lower plants. The ultimate function of these chem-

TABLE VIII

SUMMARY OF THE PURE SUBSTANCES EMPLOYED IN EXPERIMENTS ON THE GERMINATION AND GROWTH OF POLLEN TUBES, AND THEIR ACTIVITY

SUBSTANCE	SIGNIFICANT INCREASES			
	PERCENTAGE GERMINATION		TUBE LENGTH	
	TROPAEOLUM	MILLA	TROPAEOLUM	MILLA
Water soluble vitamins				
Thiamin†	.....	.....	**	.....
Niacin†	.....	.....	.....	.....
Niacinamide†	.....	.....	.....	.....
Riboflavin	.....	.....	.....	*
p-Aminobenzoic acid	.....	**	.....	*
Inositol	.....	**	.....	.....
Pyridoxin†	.....	.....	.....	**
Ascorbic acid	.....	.....	.....	.....
Ca-pantothenate	.....	.....	.....	*
α-Naphthyl acetamide	.....	.....	*	.....
Oil soluble vitamins				
2-methyl-1,4-naphthoquinone	.....	.....	.....	.....
α-Tocopherol	.....	.....	.....	.....
Plant hormones				
Indoleacetic acid	.....	.....	.....	**
Traumatic acid	.....	.....	.....	.....
Animal hormones				
Estrone	.....	.....	.....	.....
Pyrimidines and purines				
Uric acid	.....	.....	.....	.....
Adenine	.....	.....	.....	.....
Xanthine	.....	.....	.....	.....
Guanine	.....	.....	.....	**
Thymine	.....	.....	.....	*
Cytosine	.....	.....	.....	.....
Uracil	.....	*	.....	.....
2-methyl-4,6-dihydroxypurine	.....	.....	.....	.....
2-methyl-4-hydroxy-5-hydroxymethylpurine	.....	.....	.....	.....
2-methyl-4-amino-5-thioformamidomethyl-purine	.....	.....	.....	**
4-methyl-5-hydroxymethyluracil	.....	.....	.....	.....
2-methyl-4-hydroxy-5-aminomethylpurine-hydrochloride	.....	.....	.....	**
2-methyl-4-amino-5-aminomethylpurine-hydrochloride	.....	.....	.....	**
Miscellaneous compounds				
Pimelic acid	.....	.....	.....	.....
Allantoin	.....	.....	.....	.....
Alloxan	.....	.....	.....	.....
2-chloroisothiamin-iodide	.....	.....	.....	*
Acenaphthene	.....	*	.....	*

* Significant activity, less than 1 chance in 20 that the results were due to pure chance.

** Highly significant activity, less than 1 chance in 100 that the results were due to pure chance.

† These may also be classified as plant hormones.

icals within the pollen has not yet been determined, but it is reasonable to suppose that it is similar to the function of the same chemical in other parts of the plant. That is, indole-acetic acid is essential to cell elongation, and the various water soluble vitamins function in respiratory enzyme systems. The purines have been reported to promote leaf growth under some circumstances (**2**).

Even with the addition of the growth substances employed, the growth of the pollen tube can neither be considered normal nor approaching that of pollen germinated in stigma exudate. In exudate the tubes averaged 16 diameters long. In the various media employed tubes varied from 1 to 6 or 7 diameters long. Tubes in synthetic media were often thin, burst soon after germination, or were sometimes highly vesicular. This would indicate that the media employed were incomplete and lacking in some other factor(s) since it can be supplied at least in part by stigma exudate. A search is now under way for this factor(s). The results will be reported in a later communication.

Summary

This paper reports the results of tests of 33 pure growth substances on the germination of pollen and the growth of pollen tubes of Tropaeolum and Milla. Sixteen substances increased germination or tube growth significantly.

The results give evidence supporting the view that the germination of pollen and the growth of the pollen tube are at least in part physiologically independent. Certain substances may affect one process and not the other.

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AN AUTO–IRRIGATOR FOR GROWING PLANTS IN THE LABORATORY

A. D. MOINAT

(WITH ONE FIGURE)

At present, the most practical method of controlling soil moisture for the roots of laboratory plants seems to be the method of adding water to the surface soil in order to bring the soil container, its soil, and the plants growing therein to a certain predetermined weight which represents the desired moisture content. The principal difficulty in this procedure lies in attaining the original distribution of moisture in the soil which was quite uniform at first, but soon afterward becomes variable both vertically and horizontally through the soil container. HENDRICKSON and VEIHMEYER (**2**) found that uniform distribution of moisture in a soil container is unattainable after drying, until the water content again reaches the rather high level marked by the moisture equivalent. At low moisture contents, there are regions in the container with variable water content and the most active roots will be in the region of optimum moisture.

The most successful mechanism for moisture control seems to be that devised by LIVINGSTON (**3**) and further developed in a new device, the double walled pot, by RICHARDS (**4**). Here, the plants in the irrigator must pull against the weight of the column of supply water through a tube reaching to a reservoir at a lower level than the irrigator itself. In the double walled pot, RICHARDS and LOOMIS (**5**) have found a decreasing degree of control of the soil moisture the lower one departs from the moisture equivalent. At the lower moisture contents, the water is lost from the container much more rapidly than it can be replaced because of the low rate of water movement in dry soil. The soil in the container may reach the wilting percentage and the plants growing therein remain unwilted if their roots are in contact with the source of supply through the porous clay walls of the container.

In the apparatus described in the present paper, a much simpler system of water control was used, although one which has its limitations. At low moisture percentages there is difficulty in supplying moisture at a rate sufficiently high to maintain the certain desired low soil moisture which represents the actual water available to the roots of plants growing in the irrigator.

Materials and methods

The principle involved in all irrigators which attempt to control the water supply for roots, involves the separation of the roots from actual contact with the water supply. Several separating substances have been tried, such as the unglazed clay used by LIVINGSTON (**3**) and others, and the plaster-of-Paris and unglazed clay plates used in this study.

In the first trials, porous clay plates of rather uniform 3/16-inch thick-

ness, sealed with plaster-of-Paris in the bottom of tin soil containers (tops of gallon pails) were used. Nine of these were placed on the surface of sand of a rather fine textural grade, which had been uniformly compacted into a large 30 × 30-inch rectangular, galvanized iron container, 18 inches deep. The water came up from a supply in the bottom of this container, through the sand, to the porous plate in the bottom of each soil container. The water was maintained at an approximately constant level by the use of feed tubes from a flask above, using the Mariotte principle. Distribution across the bottom of the galvanized iron sand container was secured by using a layer of coarse gravel about 1½ inches deep over the bottom, upon which the sand was packed. Three levels of sand can be used in one sand container, but due to the need for more uniform light for all of the plants in one test, it is probably better to use sand at a single level in each container and that level to be near the top of the container.

Heavy 28-inch dish pans were also used as sand containers with different rates of supply secured by filling each of the several pans with one of several different textural grades of sand.

A second type of plate developed consisted of plaster-of-Paris mixed thin and poured over a compacted smooth sand in such a sand container as a glazed earthenware jar of 2- or 3-gallon capacity. Soil was then placed on the plate to fill the upper part of the jar. Great care in any case must be observed to get complete sealing of the plate with the walls of the soil container (fig. 1). The plaster-of-Paris plate formed in this fashion is not as uniform in thickness as the manufactured clay plate but has the advantage of giving excellent contact with the sand, which seemed to be a drawback of the manufactured plate.

The soil moisture has been determined by two methods: first, sampling and oven drying to constant weight at 105° C.; and, second, by use of the Bouyoucos (1) electrical resistance method. With the latter method, the plaster-of-Paris blocks in which the resistance electrodes were imbedded were laid either directly on the plaster-of-Paris plate of the irrigator or, in the majority of cases, on a thin layer of soil above the plate. The resistance was then measured with a Leeds and Northrup conductivity apparatus.

Results

Porous clay plate irrigators

The results of a series of irrigator studies using the large galvanized iron sand containers and the porous clay plates as described above, are given in table I. The sand in the irrigator was approximately 30 cm. deep. Irrigator 4 in the table was in reality a new set-up of number 3 in which the soil was replaced in the soil containers, but the containers themselves were not moved. Gradual drying of the soil containers is seen in the 3A–3C, and 4A–4B columns although this drying is not entirely uniform. It is evident from the table that there is considerable variation between the various containers of one irrigator, a divergence which is probably too great except in

studies where rather wide ranges of soil moisture are permissible. The moisture in the sand beneath the plates is also variable and often does not correlate exactly with the moisture in the soil containers above. This variation in sand moisture, which is undoubtedly not entirely due to errors in sampling, will always be a cause of some variability in moisture supply to the irrigator plates.

Stone jar irrigators and plaster-of-Paris plates

Eight stone jars were arranged as irrigators as illustrated in figure 1. Irrigator number III was set up with provision for daily reading of the electrical resistance and conditions for obtaining a gradual decline in the

TABLE I

Soil moisture in porous clay plate irrigators set up in 30 × 30-inch sand containers nos. 2–4

Soil container	Irrigator tanks						
	2A	3A	3B	3C	4A	4B	Sand*
	%	%	%	%	%	%	%
1	.....	21.5	21.0	18.3	16.9	12.0	3.46
2	13.1	19.6	17.8	16.2	16.4	12.1	3.24
3	13.4	18.9	17.9	14.7	17.6	13.3	3.56
4	11.4	18.8	18.5	17.3	18.8	15.8	3.84
5	12.8	18.0	16.8	17.4	19.4	13.2	3.97
6	13.3	18.1	18.9	15.9	17.0	12.4	3.79
7	12.4	20.17	18.7	17.9	18.3	13.0	3.77
8	14.3	20.4	20.5	18.3	19.2	15.6	4.9
9	11.2	23.5	22.3	17.9	16.3	12.1	3.34
Mean	12.7	20.2	19.2	18.7	18.1	13.3	3.77
Em†	0.40	0.58	1.76	0.31	0.39	0.49	0.17
Date started	3–16	4–7	.....	.....	5–19	.....	.....
Date sampled	4–1	5–7	5–14	5–19	5–25	6–9	6–15

* Sample taken under plates of 4B.
† Em: standard error of the mean.

moisture content of the soil. There was no disturbance of the soil for sampling until the apparatus was dismantled at the end of eleven days. Two resistance blocks were placed in the soil, one on either side of the irrigator. From table II, it can be seen that the resistance gradually increased, but somewhat irregularly. The final moisture determined from a soil sample taken from the upper surface of the two resistance blocks was 22.3 and 22.4 per cent., respectively. Although several of the irrigators in this series were set up with the view to obtaining readings at a low moisture content, none reached a low point in the time available because of the amount of water needed to pour the plaster-of-Paris plate.

Irrigator number IV was set up to run with a high moisture supply. The resistance increased in the 10-days' run rather irregularly and the moisture samples from on top of the blocks were approximately equal as is shown in table II. The resistance readings in all of the present stone-jar-irrigator

series are somewhat variable because of the high moisture in the soil. In dry soil, the end points for the hummer on the resistance apparatus are more definite.

Irrigators V, VI, and VII were rather irregular in their resistance readings, which did not always seem to follow exactly the decreases in soil moisture as determined by sampling. This irregularity may have been due to disturbance of the soil when the samples were taken, or to the fact that the resistance readings are more variable at the higher moistures.

In general, resistance readings appear to be less accurate at the higher soil moistures, and each resistance block must be calibrated in order to make

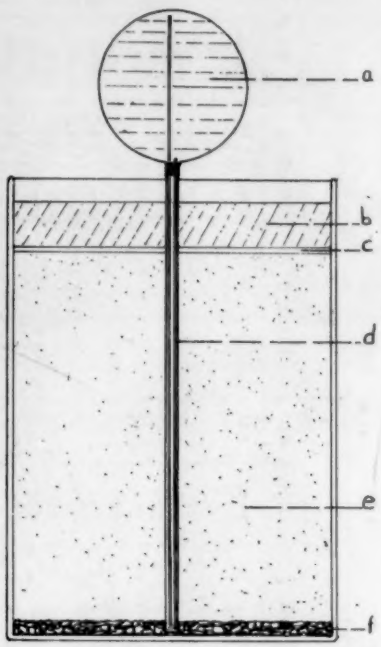

FIG. 1. Auto-irrigator with plaster-of-Paris plate: a, reservoir; b, soil; c, plaster-of-Paris plate; d, conduit for water supply tubes; e, capillary column of sand; f, gravel for distribution of water across bottom of container.

comparisons between blocks. It is believed, however, that this method has definite possibilities in certain types of work since the soil need not be disturbed while following moisture changes.

DISTRIBUTION OF MOISTURE IN THE STONE JAR IRRIGATORS USING PLASTER-OF-PARIS PLATES

From samples taken at different positions in the irrigator soil some idea of the uniformity of water supply can be gained. In general, there is slightly more water in the soil at the surface of the plaster-of-Paris plate than there is on top of the resistance block about a half inch higher in the irrigator. See table III, irrigator V.

TABLE II

ELECTRICAL RESISTANCE IN OHMS AND PERCENTAGE OF SOIL MOISTURE IN STONE JAR IRRIGATORS

DATE*	IRRIGATORS																	
	III				IV				V		VI				VII			
	Block 5		Block 4		Block 3		Block 4		Block 8		Block 6		Block 7		Block 9		Block 10	
	ohms	%	*ohms*	%	*ohms*	%	*ohms*	%	*ohms*	%	*ohms*	%	*ohms*	%	*ohms*	%	*ohms*	%
7	309	.....	510	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
7	494	.....	521	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
8	529	.....	583	.....	587	.....	583	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
8	561	.....	604	.....	609	.....	604	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
9	566	.....	611	.....	602	.....	611	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
10	589	.....	604	.....	619	.....	605	.....	560	.....	347	.....	260	.....	514	.....	599	.....
11	621	.....	688	.....	634	.....	677	.....	531	.....	398	26.7	305	25.0	553	.....	614	.....
12	559	.....	591	.....	611	.....	615	.....	500	25.8	374	24.7	276	24.7	486	29.4	490	28.2
13	579	.....	515	.....	633	.....	620	.....	477	.....	409	22.7	276	23.5	517	27.4	549	.....
14	583	.....	617	.....	653	.....	631	.....	520	.....	377	.....	288	.....	588	.....	657	.....
16	606	.....	627	.....	700	.....	666	.....	487	.....	387	23.1	300	23.86	587	26.6	710	25.4
17	595	22.3	627	22.4	666	32.2	672	32.3	496	24.6	369	22.7	293	.....	619	25.8	677	25.8
18	575	.....	631	.....	465	.....	457	.....	475	.....	363	.....	297	.....	619	.....	451	.....

* All readings were taken in August on the dates indicated.

TABLE III

VARIATION IN MOISTURE CONTENT OF SOIL AT DIFFERENT POSITIONS IN THE IRRIGATORS V–VIII

Irrigators											
V			VI			VII			VIII		
Date*	Position†	H_2O	Date*	Position	H_2O	Date*	Position	H_2O	Date*	Position	H_2O
		%			%			%			%
12	block 8	25.8	11	block 6	26.7	12	block 9	29.4	13	1	28.3
12	1, plate	26.3	12	block 6	24.7	13	block 9	27.4	13	2	27.7
13	block 8	25.4	12	3	24.2	14	2	27.3	13	3	27.4
13	3	26.4	13	block 6	22.7	16	block 9	26.6	14	2a	28.9
13	2	25.8	14	2	23.2	17	block 9	25.8	14	1a	27.3
13	2a plate	26.2	16	block 6	23.1	...	.....	.....	16	2	28.7
14	1a	22.8	17	block 6	22.7	12	block 10	28.2	16	3a	29.7
14	2	25.9	11	1a tube‡	21.5	13	block 10	28.2	...	...	.....
16	3a plate	25.1	11	3a	26.2	14	.....	26.9	...	...	.....
16	block 8	24.9	11	2a	25.0	16	block 10	25.4	...	...	.....
17	block 8	24.6	12	block 5	24.7	17	block 10	25.8	...	...	.....
17	2a plate	25.6	12	block 5	23.5	17	2	28.5	...	...	.....
			13	3	25.7						
			14	1	20.0						
			16	block 5	23.9						
			17	.....	23.0						

* All samples were taken in August on the dates indicated.

† Position, refers to the point in the irrigator where the soil was sampled. Six loci for samples were numbered in the sequence 1, 1a, 2, 2a, 3, and 3a around the irrigator about 1 inch from the outer wall. One resistance block was placed in irrigator V at position 1; two were located in irrigators VI and VII at positions 1 and 2a. Block, in the table indicates that the sample was taken from the upper surface of the block which had been given a certain number. Plate, indicates that samples were taken on the upper surface of the plaster-of-Paris plate. All other samples, except where otherwise indicated, were taken at the same level as the upper surface of the block.

‡ A full depth sample taken with a tube. All other samples taken with a spatula from a layer about ¼-inch thick.

The average difference in moisture percentages between comparable positions in irrigator VI is 0.7 per cent., while the maximum difference is 1.7 per cent. in soil of an irrigator that was rather frequently disturbed by sampling. In irrigator VII the average difference in water content of the soil from different positions was 1.05 per cent. with a maximum difference of 1.4 per cent. The average difference in irrigator VIII is 1.15 per cent. with 2.4 per cent. as the maximum. The largest differences are in the third and last sampling after some disturbance of the soil.

With the porous clay plate irrigators the average difference of comparable samples as taken from the sand containers, or tanks 2, 3 and 4 reported above, was 0.74 per cent. but was found to be over 2 per cent. in individual soil containers.

Nutrient solution in the irrigator

In stone jar irrigators numbers I and II, washed Ottawa sand was used above the plate instead of soil and the same type of sand was used for the capillary column below the plate. In irrigator I, nutrient solution was used instead of water; in irrigator II, distilled water was used for the water supply. Beans were planted in both irrigators. The plants which were grown in the irrigator supplied with distilled water for 3 weeks began to show deficiency symptoms in the form of reduced growth and lighter colored areas on the leaves. The plants which had been supplied with the same quantity of a complete nutrient solution appeared to be healthy and made good growth in spite of the fact that the nutrients had to move through the $CaSO_4$ plate. One or two small roots managed to get through the plate of each irrigator but they were too small to account for the difference in growth. Further studies will be necessary to determine the value of this method of nutrient supply.

Discussion

In the irrigator which has been described we have a simple device by means of which one can grow plants at various rates of water supply. At the lower moisture levels, the roots obtain their moisture directly from the porous plate rather than from the soil but, nevertheless, it is possible to get the desired effects on the plants. The soil over the roots, of course, does not contain as much moisture as is actually available to roots which are in contact with the plate and often massed on the plate. Covering the soil surface to prevent excessive evaporation would tend to delay the exhaustion of moisture from the irrigator soil but would also introduce aeration problems. The writer and others have used this method to obtain plants with the characteristics typically developed under different moisture supplies. In some cases florists' pots, with the drainage hole sealed with plaster-of-Paris, and then sunk in water supplying sand, have been used instead of porous plates.

Summary

1. An irrigator is described which depends upon the capillary rise of

water through sand to supply the water for plants growing in soil above a porous plate embedded in the surface of the sand.

2. By varying the depth of the sand or its textural grade, moderate sized plants can be grown under conditions of high, low, and moderate moisture supply.

3. Specifications to obtain a definite soil moisture are, for all practical purposes, impossible to list at present because of the variability in the sand, the porous separation plate, the soil, etc.

4. In a set-up of stone jar irrigators, with the soil moisture ranging between 20 and 30 per cent., the distribution of moisture across the irrigator was uniform within 2.5 per cent.

5. At the lower soil moistures, approaching the wilting point, soil samples from soil even very close to the plate may not represent the moisture available to plants with their roots in direct contact with the plate.

6. The Bouyoucos electrical resistance apparatus offers a method of following soil moisture conditions in the irrigator without disturbing the soil. The accuracy of this method proved to be less at the higher moisture contents.

The idea of the capillary column of sand and the porous separating plate for the irrigator described in this report was received from DR. C. F. HOTTES of the University of Illinois. The author also wishes to express his appreciation for suggestions and aid given by DR. S. V. EATON and MR. ROBERT WHITMORE in the course of the resistance measurements which were carried out at the Barnes Laboratory of Plant Physiology, University of Chicago.

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A METHOD OF INCREASING THE RATE OF SEED GERMINATION OF *TARAXACUM KOK-SAGHYZ*[1, 2]

J. Levitt and P. C. Hamm

(WITH THIRTEEN FIGURES)

The germination of kok-saghyz seed is a gradual process, the first seedlings pushing through in a few days, the rest appearing successively during a period of several weeks. Obviously, surface soil moisture must be maintained high throughout this period if a good stand is to be established, especially since the seeds are small and have to be sown very shallow. Since such ideal conditions seldom prevail, and since the long drawn out germination period is highly conducive to damping off, poor stands are frequently obtained. Some means of speeding up the process of germination is therefore desirable.

In view of the rapid appearance of the first few seedlings, and in the absence of hard seed coats, a plausible explanation of the tardiness of the remaining seeds is that they are not fully mature, *i.e.*, their physiological and perhaps morphological development was not completed when they were harvested.

The aim of this investigation was to find some way to mature these seeds before sowing them. This should be possible by increasing the moisture content of the seeds sufficiently to permit the necessary physiological changes, but insufficiently to permit germination. It should then be possible to dry the seeds so that they can be sown in the usual way.

The two recommended methods (presoaking and vernalization) wittingly or unwittingly make use of this principle. The former, however, permits some germination, and in both cases the seeds are sown wet. Consequently injury sometimes results. Furthermore, it is difficult to obtain uniformity in water uptake by these methods. Some better procedure must therefore be sought.

In the following investigation, unless otherwise stated, each value for germination is an average of three Petri plates containing 100 seeds per plate. The seeds were sown on a piece of filter paper which covered the bottom of the plate, and were supplied with 5 ml. of water. Since temperature was not controlled (except when stated), individual experiments cannot be compared directly with each other.

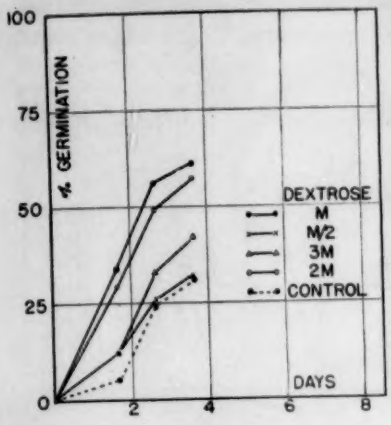
The amount of water absorbed by the seeds can be accurately controlled by permitting the seeds to come to equilibrium with a solution of known concentration. Seeds were therefore soaked in dextrose solutions of different concentrations, rinsed with water, dried for about 8 hours, and sown. The stimulation was distinct and none of the seeds were injured by the treatment

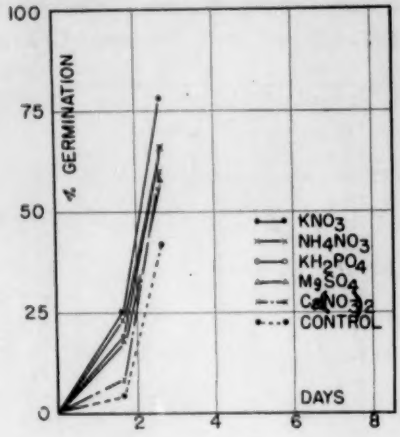
[1] The seeds used in this investigation were obtained from the U.S.D.A.

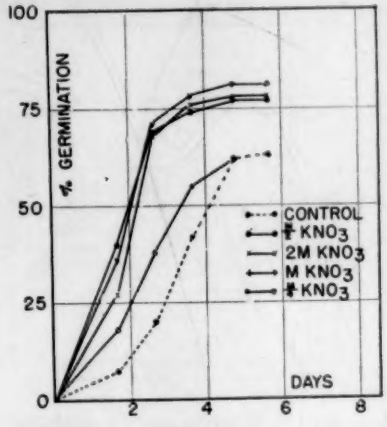
[2] Paper no. 2052, Scientific Journal Series, Minnesota Agricultural Experiment Station.

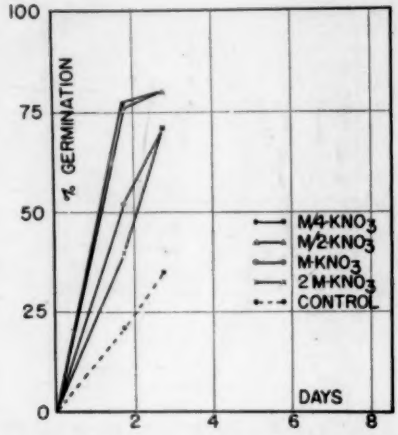
(fig. 1). Not all of the concentrations tried were of equal value. There was an optimum range, (M dextrose), on either side of which the effect decreased.

If the effect of the solution is simply osmotic, it should be possible to









FIGS. 1–4. Upper left: Seeds soaked for 3 days in dextrose solutions, rinsed, dried for 8 hours, then sowed.

Upper right: Seeds soaked for 3 days in salt solutions, rinsed, dried for 8 hours, then sowed.

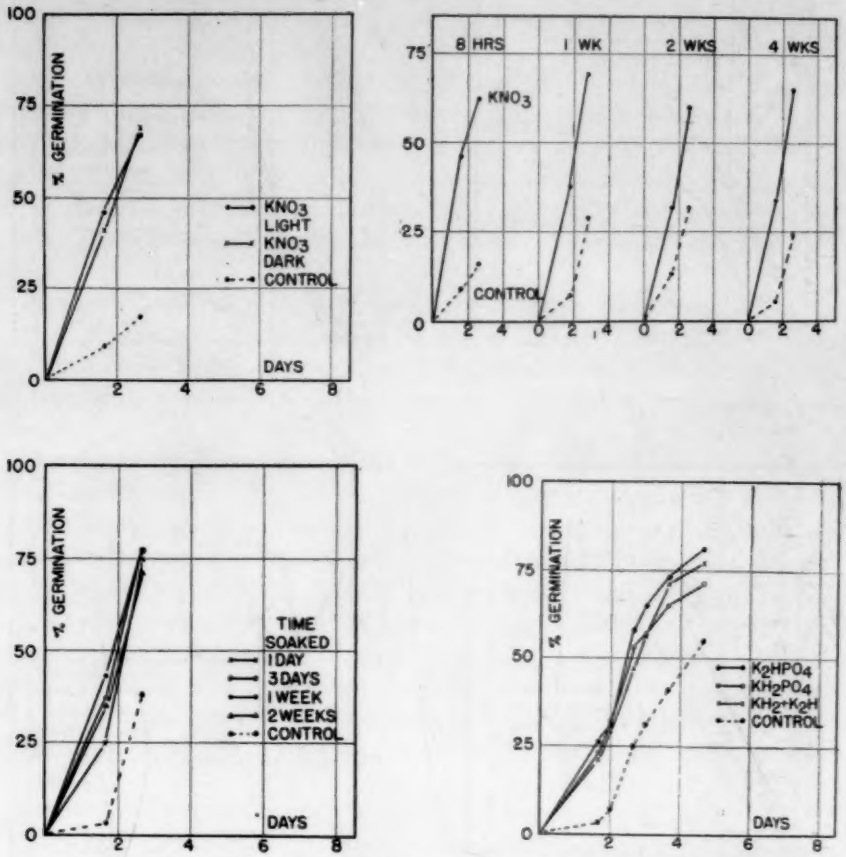
Lower left: Seeds soaked for 3 days in KNO_3 solutions, rinsed, dried for 8 hours, then sowed.

Lower right: Seeds soaked for 3 days in solutions, rinsed, then sowed without previous drying.

duplicate these results with other solutes. This would be desirable since even the stronger dextrose solutions become mouldy. Mineral nutrients were next tried, in the hope that they might also have a stimulating effect on the seedlings due to their nutrient value.

All the solutions tried increased the rate of germination, some more than others (fig. 2). The differences are not necessarily due to the chemical nature of the solutes, since though the molar concentrations were the same, the osmotic values were not.

Decreasing the concentration increased the stimulation up to a point (fig. 3), just as in the case of dextrose. Apparently, if the water content



FIGS. 5–8. Upper left: Seeds soaked for three days in M KNO_3 one set in the light, the other in the dark. Both rinsed, dried, then germinated in the dark.

Upper right: Seeds soaked for 3 days in M KNO_3, rinsed, dried for 8 hours, 1 week, 2 weeks, and 4 weeks, respectively, then sowed.

Lower left: Seeds soaked in M KNO_3 for 1 day, 2 days, 1 week, and 2 weeks, respectively, then rinsed, dried 8 hours, and sowed.

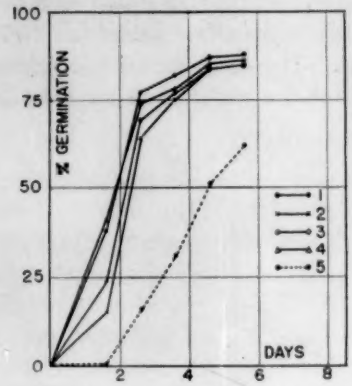
Lower right: Seeds soaked in M KH_2PO_4, M K_2HPO_4, and a 1: 1 mixture of the two, respectively, for 3 days, then rinsed, dried, and sowed. pH's of solutions, 4.1, 8.9, and 7.6.

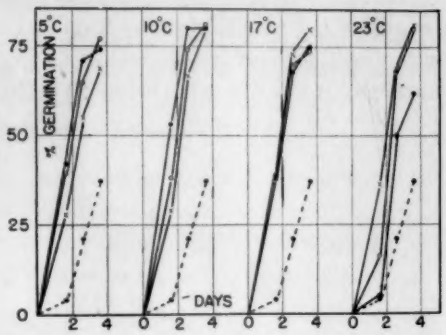
of the seeds is increased too much, the effect is lost during the subsequent drying process, even though no sign of germination is evident. When the same experiment was repeated without drying the seeds, the lowest concentration produced the greatest stimulation (fig. 4).

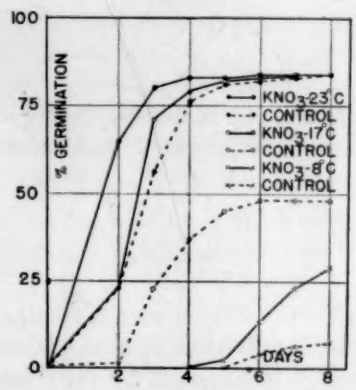
The effect of other factors on the stimulation produced by the osmotic method was next investigated. Treatments were found to have the same effect whether conducted in the light or in the dark (fig. 5).

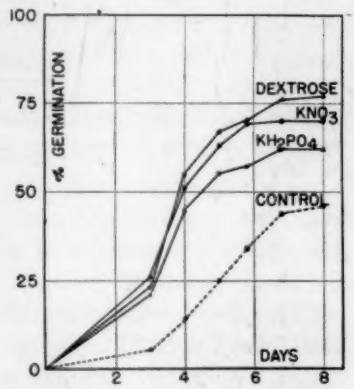
From the practical point of view, it is of prime importance to know whether the effect is retained for long periods after drying. Figure 6 shows that it is.

Surprisingly short soaking periods proved to be as effective as considerably longer ones (fig. 7). The longest periods seemed somewhat less effective.









FIGS. 9–12. Upper left: Seeds soaked 3 days in M KNO_3, then rinsed once, (1); sowed without rinsing (2); washed for two hours (3); and rinsed three times with 10 ml. water (4). All were then dried and sowed. Untreated control (5).

Upper right: Seeds soaked in M KNO_3 at 5° C., 10° C., 17° C., 23° C., then rinsed, dried and sowed. ×———×, soaked 4 days; ○———○, soaked 1 week; ●———●, soaked 2 weeks.

Lower left: Seeds soaked 3 days in M KNO_3, rinsed, dried, then sowed at 23° C., 17° C., 8° C., respectively. Similar results were obtained at 4° C., germination beginning after 2 weeks.

Lower right: 100 seeds soaked 3 days in M dextrose, M KNO_3, M KH_2PO_4 respectively, then rinsed, dried, and sowed in soil (about $\frac{1}{8}$–$\frac{1}{4}$ inch deep).

It was thought that since pH affects the hydrophily of colloids, this factor might have some influence on the degree of stimulation. Monobasic and dibasic potassium phosphate were therefore compared with each other and with mixtures of the two. The differences were slight (fig. 8).

The amount of washing after soaking had very little effect when KNO_3 was the solute used (fig. 9). More toxic solutes, however, (*e.g.* $MgSO_4$, NH_4NO_3) must be removed more thoroughly, or the effect is lost and even a retardation of germination, or death of the seeds, may result.

For practical as well as theoretical reasons, it is important to know the relationship of the stimulation to temperature. Figure 10 shows that the length of the soaking period required to produce the effect varies inversely with the temperature, as would be expected. The temperature coefficient, however, is low enough (below 2) to indicate that a physical factor is limit-

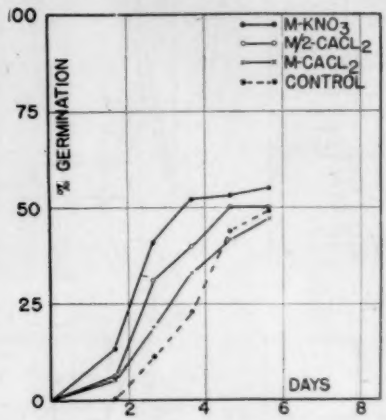


FIG. 13. Seeds exposed to atmospheres in equilibrium with M and M/2 $CaCl_2$ for 1 week, then sowed at the same time as control set and set soaked 3 days in M KNO_3, rinsed and dried.

ing. It is interesting to note that at 23° C. the 4-day treatment is best, at 5 and 10° the 2-week period is best, at 17° C. the three lengths of treatment had the same effect.

The effect persists regardless of the temperature during germination, at least from 4 to 23° C. (fig. 11). One surprising difference is, however, revealed. At 23° C. only the rate of germination is increased by the treatment, at 17° C. both the rate and the total percentage of germination, at least at the end of 3 weeks.

All these tests were performed in Petri plates. It is, of course, essential to know whether the same effect is obtained in soil. Figure 12 shows that it is.

Some other kinds of seeds were tested to determine whether the stimulating effect of the osmotic method is specific to kok-saghyz.

Kentucky bluegrass seed was soaked for 8 days in M KNO_3, washed, then dried before sowing. Little or no germination occurred in either the treated

or the control seeds, as long as they were kept in the dark. On transfer to the light, however, the treated seed germinated to the extent of 98 seeds in 300, the control 7 seeds in 300, in a period of one month. Rutabagas, on the other hand were apparently injured by the treatment and germination was markedly reduced.

If the osmotic method owes its effectiveness simply to an osmotic control of the amount of water taken up by the seeds, similar results should be obtainable by simply exposing the seeds to an atmosphere of the same vapor pressure as the solution. Seeds were therefore exposed to the atmosphere over solutions of $CaCl_2$ for a week, then sown (fig. 13). This method induced a stimulation, though not as great as that obtained by the osmotic method. The difference may perhaps be due to slowness of absorption of water vapor.

Summary

Germination of kok-saghyz seed was hastened by permitting the seeds to absorb water in quantity insufficient to induce germination, then drying the seed before sowing. The stimulation was obtained whether the uptake of water was controlled osmotically or by exposure to atmospheres of definite relative humidity.

The following results were obtained with the osmotic method. No loss of the stimulation occurred even 4 weeks after drying. The optimum concentration was M/2 for KNO_3, M for dextrose. When the seeds were sown without being dried, even weaker concentrations were optimum. Two weeks' treatment proved better than shorter periods at 5 and 10° C., 4 days was better than 1 or 2 weeks at 23° C., whereas at 17° C. all three lengths of time gave the same results. Two weeks at 10° C. gave the best results of all the above. The Van't Hoff coefficient was less than 2. When germinated at 23° C., the treatment had no effect on the final percentage of germination. At lower temperatures it increased the final percentage of germination. Factors having little or no effect on the stimulation are: the nature of the solute (if thoroughly washed off), the amount of washing (in the case of KNO_3), the pH of the solution, the presence or absence of light.

The stimulation was obtained whether the seeds were germinated in Petri plates or in crocks of soil.

INHIBITING INFLUENCE OF THE LEAVES ON THE PHOTOPERIODIC RESPONSE OF NOBEL SPINACH

ALICE P. WITHROW, R. B. WITHROW, AND J. P. BIEBEL

(WITH TWO FIGURES)

Introduction

CAJLACHJAN (**2**), MOSKOV (**9**), LUBIMENKO (**8**), HAMNER and BONNER (**3**), HAMNER and LONG (**4**) and BORTHWICK and PARKER (**1**) have shown that the flower forming stimulus in short day plants is initiated in the leaves. One leaf of a normal plant kept in a favorable short photoperiod was reported to be sufficient to induce flowering in the short day plants, Biloxi soybean (**1**) and Xanthium (**3**), even though the remaining leaves on the plant were kept in an unfavorable photoperiod. Under certain circumstances, however, involving two-branched plants or grafted plants where one branch or plant is induced to flower while the other is kept in an unfavorable photoperiod, the leaves on the latter branch or plant have been shown to inhibit flowering of that branch or plant. The effect is not carried over to other plant portions (**1, 3, 5**).

Work of this type on long day plants has not been very extensive. KNOTT (**6**) reported that the flower stalk formation in Virginia Savoy and Old Dominion varieties of spinach was not affected by the photoperiod given the bud but only by that received by the leaves. LANG and MELCHERS (**7**) reported that leafy biennial *Hyoscyamus niger* plants which were pre-treated with a low temperature of 5° C., flowered only in a long photoperiod and failed to flower in a short photoperiod or in darkness. If, however, all the leaves were removed from plants which had received a low temperature treatment, they flowered under long photoperiods, short photoperiods, or in darkness. This indicates that the failure of this species to flower in a short photoperiod or in darkness may be caused by an inhibiting effect exercised by the leaves.

Methods and results

PRELIMINARY EXPERIMENTATION

A preliminary experiment with Nobel spinach was run to determine the reactions of this variety to photoperiodic treatment of the bud and the leaves. The plants were grown in flats of a sand-peat mixture and were supplied with a complete nutrient solution. Vegetative plants which had been kept in a 10-hour photoperiod from germination were used for these and subsequent studies. A night temperature of 60° F. was maintained with a minimum day temperature of 70° F. Part of the plants were left intact and part were defoliated so that only the last matured leaf remained. The defoliation

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technic was used when later experimentation showed that leaves in an unfavorable photoperiod exercised an inhibiting influence on flower bud initiation. It was thought desirable to reduce the number of such leaves to a minimum.

The intact plants were given two treatments, 24 plants per treatment, using essentially the same technic as that described by KNOTT (**6**). In the first treatment, the plants were covered with opaque rubberized black cloth so as to maintain a 10-hour photoperiod. The covering was perforated with holes 16 mm. in diameter with iron washers centered over the holes to help hold the cloth in place. The holes were so placed as to expose the bud and a few adjacent young leaves. The plants were then placed under a 24-hour photoperiod (continuous irradiation) made up of solar radiant energy supplemented with 20 footcandles of incandescent lamp radiant energy. This was used for all subsequent long photoperiod treatments. The buds and a few adjacent young leaves, therefore, received 24-hour photoperiods while the remainder of the plant received 10-hour photoperiods.

The second treatment was begun 38 days later, using vegetative plants of the same age as in the first treatment. The plants were placed under the long photoperiod with the entire plant exposed. Final data were taken 52 days after the first treatment was begun.

The one-leafed plants received three treatments, 16 plants per treatment: (1), the entire plant was given a 24-hour photoperiod; (2), the bud was given a 10-hour photoperiod and the leaf a 24-hour photoperiod; and (3), the bud was given a 24-hour photoperiod and the leaf a 10-hour photoperiod. Individual squares of opaque rubberized black cloth were so arranged that the indicated portions of the plants received the indicated photoperiod. Dissection under a low power binocular microscope was made at the end of the experimental period where macroscopic buds were not visible.

No intact plants formed flower buds except those in which the leaves received a long photoperiod, confirming KNOTT's results with Virginia Savoy and Old Dominion varieties of spinach. Where the plants were defoliated to one leaf and the bud was given a long photoperiod while the leaf received a short photoperiod, the plants also failed to form flower buds. If only the leaf received the long photoperiod and the bud was given a short photoperiod, the results were the same as when both the leaf and bud were given a long photoperiod, *i.e.*, all the plants flowered.

INHIBITING INFLUENCE OF THE LEAVES

In an endeavor to determine if the presence of leaves in an unfavorable photoperiod inhibits flower bud initiation, the flowering of intact plants with only a portion of the plant in a favorable photoperiod and of partially defoliated plants was studied.

Intact plants with approximately nine leaves were given three treatments. A number of leaves developed during the experimental period,

increasing the initial number. The treatments were: (1), one leaf exposed to a 24-hour photoperiod while the remainder of the plant was given a short photoperiod by covering with opaque rubberized cloth at the end of 10 hours of solar irradiation; (2), three leaves exposed to a long photoperiod with the remainder in a short photoperiod; (3), the entire plant exposed to a long photoperiod. The method of curtaining the plants is shown in figure 1.

The plants were grown in subirrigation gravel culture with a complete nutrient solution. The minimum night temperature was approximately 60° F. with a minimum day temperature of 70° F. Twenty-four plants were used for each treatment. The long photoperiod was applied for 26 days after which time the plants were harvested. The plants were 58 days old at the beginning of the experiment. Microscopic dissection was made to observe floral primordia at the close of the experiment.

FIG. 1. Method used to curtain intact plants so that one and three leaves received a 24-hour photoperiod while the remainder of the plant received a 10-hour photoperiod.

No flower primordia could be found at the end of the treatment when only one leaf received a long photoperiod. When three leaves were given a long photoperiod, no macroscopic buds developed; but on microscopic examination, 70 per cent. of the plants were found to have developing floral primordia. If the entire plant received a long photoperiod, all the plants had macroscopic buds at 17 days; the first buds were visible in 12 days.

In the defoliation experiments, plants with about nine leaves were defoliated respectively to one and three leaves and left intact. The leaves left on the plant were the youngest mature leaves and were more or less symmetrically arranged about the bud when three remained. The older leaves and the young developing leaves were removed. Young developing leaves were removed daily from the partially defoliated plants after the beginning of the experimental treatments. The plants were placed in a 24-hour photoperiod immediately after defoliation. The same number of leaves received the long photoperiod as was the case with the intact plants. The cultural conditions, age and number of plants, selection of leaves for the

long photoperiod treatment, length of treatment, and method of observation for flower buds were the same as for the intact plants.

The plants defoliated to one and three leaves all had macroscopic flower buds 19 days after the beginning of the treatment, about two days later than did the intact plants. The plants with one leaf first showed floral buds 16 days after the beginning of the treatment and those with three leaves, 13 days. The rate of flower stalk development was greater, in direct proportion to the number of leaves on the plant. It should be pointed out that the leaf area eventually exposed to the long photoperiod was not in proportion to the number of leaves since those plants having only one or three leaves produced much larger leaves than did the intact plants. Figure 2 shows plants in which one and three leaves of partially defoliated and intact plants received 26 long photoperiods.

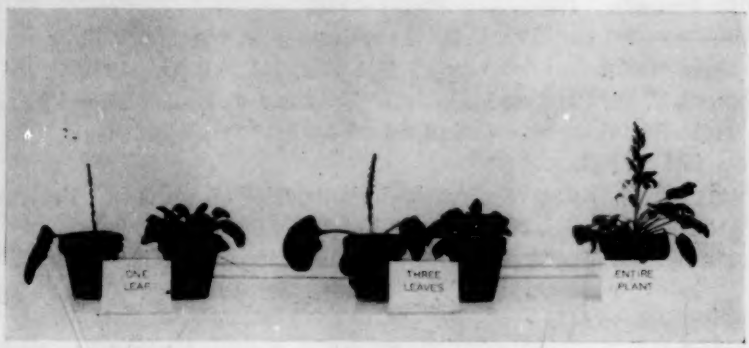

FIG. 2. Flower stalk formation resulted when Nobel spinach plants were defoliated to one and three leaves and given a long, 24-hour photoperiod treatment (left plants of each pair). When corresponding numbers of leaves of intact plants were given long photoperiod treatments (right plants of each pair), those with one leaf exposed to the long photoperiod failed to form floral primordia. Those with three leaves exposed formed floral primordia but no flower stalks during the experimental period.

Discussion

The results secured indicate that the bud of Nobel spinach is relatively insensitive to photoperiod and that the phasic development of the bud is controlled principally by reactions in the leaf. Unlike Biloxi soybean (**1**) and Xanthium (**3**), one leaf of a normal intact plant exposed to a favorable photoperiod was not sufficient to cause flower bud initiation if the remainder of the plant was given a short photoperiod. One leaf, however, was sufficient in the absence of leaves in an unfavorable photoperiod, indicating that leaves in an unfavorable photoperiod exert an inhibiting influence on flower bud initiation and development. Even if three leaves, which approximated one-third of the leaves of the plant at the beginning of the treatment, were exposed to a long photoperiod treatment, the remainder of the leaves kept in an unfavorable photoperiod exerted an inhibiting influence on the expression of the flower forming stimulus initiated in those leaves which were in a long photoperiod.

Conclusions

1. The initiation of flower buds in Nobel spinach is controlled by the leaf and the bud is relatively insensitive to photoperiod.

2. Leaves kept in an unfavorable short photoperiod exert an inhibiting influence on flower bud initiation and development in Nobel spinach.

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THE AMERICAN MISTLETOE WITH RESPECT TO CHLOROPHYLL AND PHOTOSYNTHESIS[1]

R. O. Freeland

(WITH ONE FIGURE)

Introduction

In numerous botanical texts one may find a paragraph or so devoted to mistletoe. The context is very much the same whether the books are of old or recent vintage. The authors usually state, among other things, that mistletoe is parasitic upon the host plant for water and minerals but may be only partially parasitic with respect to food since it contains an abundance of chlorophyll and can make its own food.

It is obvious that American mistletoe, *Phoradendron flavescens,* is attached to the host and that it contains green plastids. Heinricher (**2**) found that the green pigment did not develop in mistletoe in the absence of light. But the author has never seen any published evidence that this group of plants can carry on photosynthesis or that the green pigment is chlorophyll. It is felt that the data included in this paper might help to substitute facts for speculation. It is also hoped that someone living in an area where mistletoe is common may follow up this work with ringing, dark-box, and other experiments which may clarify the problem of relative dependence or independence of mistletoe with respect to the host plant.

Green pigments

In the preparation of the leaves of mistletoe for extraction they were first dried at 80° C. and then powdered. The pigments were dissolved from 5 gm. of the leaf powder with warm 95 per cent. ethyl alcohol. The alcohol plus the pigments was then diluted to 82 per cent. with water. To this latter solution 200 ml. of petroleum ether were added. After being shaken the green pigments largely separated with the petroleum ether layer. Then to this ether fraction with the green pigments an equal volume of 92 per cent. methyl alcohol was added. After being shaken together these two solvents separated into two layers, each containing a fraction of the green pigments.

Each of the two solutions obtained was quite green in transmitted light and showed red fluorescence in reflected light. By trial it was found that one-half centimeter depths of each solution gave the most distinct differences in light absorption. So using this depth of solution and solvent, respectively, the extinction coefficients of each of these fractions were determined at short intervals in the visible portion of the spectrum. The results are recorded in table I and figure 1.

[1] Paper from the Department of Botany, Northwestern University.

TABLE I

EXTINCTION COEFFICIENTS (NEGATIVE LOGARITHM OF THE PERCENTAGE OF LIGHT TRANSMISSION) FOR SOLUTIONS OF GREEN PIGMENTS OF AMERICAN MISTLETOE LEAVES. (A) THE PETROLEUM ETHER FRACTION; (B) THE METHYL ALCOHOL FRACTION

SPECTRUM	EXTINCTION COEFFICIENT		SPECTRUM	EXTINCTION COEFFICIENT	
	A	B		A	B
mμ			mμ		
440	1.28	2.42	590	0.20	0.27
450	1.24	1.90	600	0.32	0.43
460	1.20	1.95	610	0.29	0.40
470	1.12	1.95	620	0.31	0.40
475	.....	1.61	625	.....	0.50
480	0.69	1.12	630	0.39	0.58
485	.....	0.65	635	0.67	.....
490	0.34	0.39	640	1.22	1.04
495	.....	0.27	645	.....	1.33
500	0.19	0.19	650	1.70	1.40
510	0.09	0.15	655	1.11	1.18
520	0.09	0.10	660	0.52	0.83
530	0.13	0.14	670	0.12	0.14
540	0.08	0.13	680	0.05	0.12
550	0.02	0.14	690	0.02	0.03
560	0.12	0.12	700	0.07	0.06
570	0.11	0.21	710	0.07	0.03
580	0.18	0.23	720	0.07	0.03

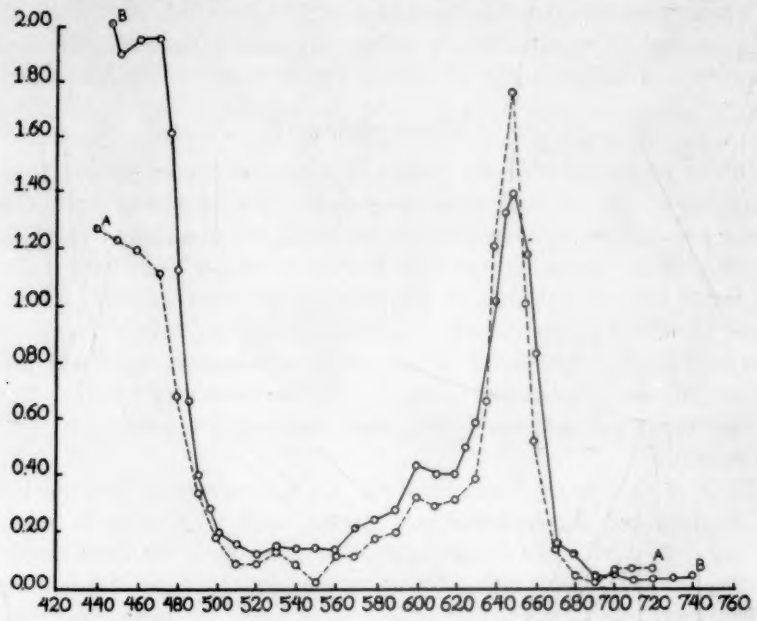

FIG. 1. Curves for light absorption for solutions of the green pigments of American mistletoe leaves. A, petroleum ether fraction; B, methyl alcohol fraction. Extinction coefficient values on the ordinate. Wave length of light in mμ on the abscissa. From data in table I.

From what is known of the relative solubility of the chlorophylls, chlorophyll *a*, if present, should be in the petroleum ether fraction and chlorophyll *b* in the methyl alcohol. From the tabulated results it is clear that the green pigments in the methyl alcohol gave strong absorption bands at 440–445 and 645–655 mμ with lesser ones at 530 and 600–620 mμ; whereas the principal absorption bands for the pigments in the petroleum ether fraction were at 440–470 and 640–655 with lesser ones at 530, 560, and 600 mμ. The absorption bands for the two solutions are somewhat similar (fig. 1), except that the principal bands for the petroleum ether fraction are shifted toward the red end of the spectrum as compared with the methyl alcohol fraction.

A comparison of these results with the known absorption bands for chlorophyll leads the writer to conclude that chlorophyll *a* and chlorophyll *b* were present in the petroleum ether and methyl alcohol, respectively.

Photosynthesis

Determinations of carbon dioxide used or produced by mistletoe were made during the months of March, April, July, and August. Short sec-

TABLE II

DATA RELATIVE TO THE RATES OF PHOTOSYNTHESIS IN THE AMERICAN MISTLETOE, BASED UPON THE AMOUNT OF CO_2 USED. FROM 25 TO 50 CC. OF MISTLETOE WERE USED IN EACH EXPERIMENT

TRIAL	TEMPERATURE	TIME	CARBON DIOXIDE CONSUMPTION*	
			APPARENT	CALCULATED TOTAL
	C.°	*hr.*	*mg.*	*mg.*
1	29–33	2.5	0.0	1.8
2	32–37	3.0	1.5	4.5
3	29–33	2.5	5.0	6.8
4	33–38	3.0	4.0	7.0
5	25–37	3.0	0.0	2.0
6	36–35	3.5	0.0	3.3

* Milligrams CO_2 per 10 ml. mistletoe per hour.

tions of the host tree branches were sawed off, leaving the clumps of mistletoe intact. The amputated stems were submerged in water with the parasitic plants in the air. Under these conditions the plants were kept in the greenhouse and showed no signs of deterioration in the few days while they were being used. During the experiments the plants were inclosed in bell jars, through which a continuous flow of air passed, before going through absorption towers containing potassium hydroxide solution after the manner of HEINICKE and HOFFMAN (1). Thus changes in carbon dioxide were measured under the conditions of light and temperature incident in the greenhouse and then with the belljar covered with a light-proof hood. These latter measurements of respiration were considered advisable because of the rather large bulk of host stem in the experimental chambers. Although

the rates of respiration were probably not the same in the light and dark, the amount of apparent photosynthesis was corrected for these rates of respiration in an attempt to find the approximate total photosynthesis. The rates were all calculated on the basis of the total volume of leaves plus stems of mistletoe, since both are green. Obviously the values would be larger if only the leaves had been used as a basis for calculation.

Many determinations were made under different light intensities and temperatures such as normally occur from day to day in a greenhouse. The results of a few of these separate trials are given in table II. It will be noted that the values for apparent photosynthesis vary from zero to positive. Some of the low values for apparent photosynthesis were undoubtedly due to the large amount of respiration found for the section of host stem and mistletoe plants. The approximate total photosynthesis (apparent photosynthesis corrected for respiration) was always positive; therefore, the data indicate that this species of mistletoe can and does carry on photosynthesis.

Summary

1. The American mistletoe, *Phoradendron flavescens,* was examined with respect to the identification of the green pigments and possible photosynthesis.

2. Extinction coefficients for the green pigments indicate the presence of chlorophylls *a* and *b*.

3. Photosynthesis was found to occur in this species of mistletoe.

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BRIEF PAPERS

CHLOROSIS IN SUDAN GRASS[1]

F. L. Wynd

Introduction

Sudan grass is one of the most important forage crops in the Rio Grande Valley, Texas. Growth is luxuriant and rapid, and the high yields suggest that the nutrients in the soil are eminently satisfactory for the growth of this species. Repeated cuttings may be made, often at intervals as short as 8 to 10 days, until 3 to 10 crops have been harvested. The leaves from the latter crops, however, become progressively more chlorotic until they become almost devoid of chlorophyll. Sometimes chlorosis appears in the second cutting, in which case the plants rarely survive more than 3 harvests. Other plantings do not become chlorotic until the third or even the fourth harvest, and in these instances the plants survive for a longer period. This chlorosis is one of the most serious diseases of Sudan grass in this area, and it often becomes a limiting factor in its production. For the past 3 years, the author has carried out field experiments in the vicinity of Elsa, Hidalgo County, Texas, in an effort to discover the cause of this nutritional disturbance. These experiments were designed particularly to test the validity of several current theories concerning the causes of this disease. The study is incomplete, but the imminence of the writer's induction into military service and the importance of the problem itself has induced him to publish a brief statement of the present status of the study in the hope that it will aid students of the general problem of nutritionally induced chlorosis.

Experimentation

Effect of soil reaction

All of the soils of this area belong to the pedocal group. The pH values of water extracts vary from 7.2 to 8.2, depending on the amount and nature of the bicarbonate and carbonate salts present. This suggests that the alkalinity of the soil may cause chlorosis by rendering iron, and other trace elements, unavailable. The fact that the chlorosis of citrus trees in the area frequently disappears if the soil is treated with acidifying agents lends credence to this theory. Accordingly, field plots were treated with various amounts of ammonium sulphate, ammonium phosphate, and sulphur, and the results were observed over a period of 3 successive seasons. Although the pH of the upper layer of the soil was changed in degrees varying from 8.2 to 3.5, no effect on the chlorosis of Sudan grass was observed.

Effect of ferrous sulphate

A set of plots were arranged adjacent to those described above and vari-

[1] The expenses incurred in the present study were borne in part by a grant from the Cerophyll Laboratories, Inc., Kansas City, Missouri, to the Graduate School of the University of Illinois.

ous amounts of ferrous sulphate were added in addition to the acidifying agents. Chemical analysis of the leaves showed that this caused an increased absorption of iron, but in no case was there any lessening of chlorosis.

Effect of applying trace elements in sprays

Dilute solutions of iron, manganese, copper, zinc, and boron were applied singly and in combination by a hand spray. Applications were made twice to each crop, but this did not prevent chlorosis.

Effect of the calcareous layer in the profile

It seems significant that the first crop produced is never chlorotic and it shows no sign of nutritional disturbance. This suggests that the roots are feeding in the upper, less calcareous layers during the early period of growth, but as the root system expands with age, they enter the lower, highly calcareous zone. The fact that the roots tend to follow the lowering zone adequately supplied with water tends to accentuate this downward penetration of those roots most active in absorption. Highly calcareous clay occurs at a depth of 24 to 36 inches in the soils which were included in the present study. This layer is rich in nutrient ions, but its high pH would seriously affect the availability of iron and other trace elements, hence the aerial growth produced during this period of growth might suffer from a lack of these substances. This theory was tested by carefully dissecting the soil profile at the time the first harvest was made, and before chlorosis had appeared. Even at this early age, when the plants showed no sign of nutritional disturbance, the roots had penetrated as deeply as 45 inches which was well into the layer of carbonate accumulation.

Effect of calcareous clay

The experiment described above is not conclusive, since significant absorption undoubtedly occurred in the upper layers of the soil profile which were sometimes only slightly calcareous. A supply of highly calcareous clay from the lower levels of the profile was removed and mixed in various proportions with the top soil. In no case, did chlorosis appear earlier than in the plants growing in the undisturbed natural soil.

Effect of repeated harvests in other areas

It might be argued that the roots of Sudan grass are intrinsically unable to maintain a normal supply of nutrients to successive growths of tops. The roots and tops may become out of "balance" in some physiological relationship under the abnormal condition imposed by frequent removal of the tops. This theory was tested by growing Sudan grass near Midland, Douglas County, Kansas, on soils of varying base exchange capacity, and which contained various amounts of carbonates. Although some of these soils contained approximately the same amount of total replaceable bases as did the soils of Hidalgo County, Texas, and also sufficient carbonates to give

a soil reaction of pH = 8.0, the growth from successive cuttings did not become chlorotic.

Effect of the soil itself

The results of the experiments described above suggest that there is some nutritional phenomenon associated with the intrinsic nature of the soils of the Lower Rio Grande Valley that induces chlorosis of Sudan grass. This possibility was tested by transporting topsoil from 10 of the most important soil series of Hidalgo County to Urbana, Illinois, where pot studies were conducted in the greenhouse. Six successive cuttings were taken during the summer and early autumn of 1942, and in no instance did chlorosis appear!

Discussion

The experiments described in this paper indicate, superficially, that the climate, and not the nutritional aspects of the soils of southern Texas produces chlorosis in the later cuttings of Sudan grass. Although the various aspects of climate undoubtedly affect the physiology of absorption, it seems more reasonable to suppose that climate is secondary to the effect of some, as yet unrecognized, aspect of the nutritional environment presented by the soil in producing the chlorosis described. There is no evidence at the present time concerning these causative factors.

Conclusions

Although Sudan grass grows luxuriantly in southern Texas, successive cuttings become progressively chlorotic. The plants finally become so devoid of chlorophyll that further growth is impossible. This condition is not due to the pH of the soil, to the availability of trace elements, to calcareous layers in the soil, nor to any intrinsic inability of the root to support continued vegetative growth. Climatic factors may prevent the appearance of chlorosis, although it is probable that climatic effects are secondary to some unknown nutritional property of the soil profile.

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ENZYME ACTION DOMINATED BY ASSOCIATED COLLOIDS

H. C. EYSTER

Recently EYSTER (1) showed that the adsorptive capacity of finely ground activated charcoal for methylene blue was reduced by ether, chloroform, sodium barbital, sulfanilamide, and saponin as well as by ethyl alcohol. This research was promoted by JOHNSON, BROWN and MARSLAND (3) who reported that luminous bacteria were partly or totally "blacked out" by the application of narcotics. In addition, EYSTER investigated the action of diastase on soluble starch and found that it, too, was reduced by the same narcotics. It was assumed that the narcotics decreased the adsorptive capacity of the enzyme for the substratum in a manner similar to the effect of narcotics on activated charcoal. The logical conclusion was that the results supported the argument that enzyme action is fundamentally adsorptive. This would then mean that the effect of narcotics on luminous bacteria was simply a reduction in the adsorptive capacity of the enzyme, luciferase.

Subsequently, GEIGER (2) cast doubt on the validity of those experiments for satisfactorily explaining the mechanism of narcosis of luminous bacteria. He based his opinion on the fact that "narcotics which retard the action of isolated diastatic ferment markedly increase the diastatic activity of liver cells." This instigated an investigation to bring about a correlation of these two apparently conflicting sets of observations.

The experiment described in table I was performed. In every case 50 ml. of 1 per cent. soluble starch, 5 ml. of a 1 per cent. diastase solution, and enough narcotic to give the stated concentration were diluted to 100 ml. with distilled water. The concentration for each narcotic was based on the final solution volume of 100 ml. The charcoal was kept dry and carbon-dioxide-free in a desiccator with $CaCl_2$ near the top and KOH on the bottom. The temperature of the digestive mixtures was kept at approximately 25° C.

As the table readily shows, ethyl alcohol, ether, and chloroform decreased the digestive action of diastase on soluble starch when the diastase was not associated with charcoal. When associated with charcoal, however, the digestive action was increased as reported for the liver cells. It appears that the charcoal adsorbs the enzyme and prevents it from acting freely on the soluble starch. It also appears that the narcotics, being more drastic in their effect on the adsorptive capacity of charcoal than on the adsorptive capacity of enzymes, release the enzyme from the charcoal particles and give it greater freedom to digest the soluble starch.

Sulfanilamide presented a different picture. While ethyl alcohol, ether, and chloroform markedly increased the activity of diastase when they were associated with finely ground charcoal, sulfanilamide did not alter it appreciably. The effect, if any, was a very slight increase in the rate of diastatic activity. This is commensurate with the effect of sulfanilamide

TABLE I

THE EFFECT OF SOME NARCOTICS ON THE DIGESTIVE ACTION OF DIASTASE IN PRESENCE OF CHARCOAL

NARCOTIC, WITHOUT CHARCOAL	MINIMUM TIME FOR SOLUBLE STARCH SOLUTION TO BE DIGESTED PAST THE LAST IODINE STAINING STAGE
	min.
Control (no narcotic added)	15
Ethyl alcohol, 25 per cent.	30
Ether, 25 per cent.	17
Chloroform, 25 per cent.	25
Sulfanilamide, 0.2 per cent.	19
NARCOTIC, WITH 1 GM. CHARCOAL	
Control (no narcotic added)	234
Ethyl alcohol, 25 per cent.	35
Ether, 25 per cent.	17
Chloroform, 25 per cent.	53
Sulfanilamide, 0.2 per cent.	205

on the action of diastase in the absence of charcoal; *i. e.*, sulfanilamide reduces diastatic activity very slightly as shown by EYSTER (11). The fact that sulfanilamide did not alter, or only slightly increased, the activity of enzymes adsorbed on charcoal may be another part of the reason why sulfa drugs are so effective in controlling bacterial diseases.

These findings are intensely significant because they indicate that diastase in liver cells is adsorbed on cellular colloids such as proteins, and that all enzymes in whatever cell may be similarly associated with colloids. In a study of enzymes it is well to bear this in mind and to make our interpretations accordingly. The influence of a factor on isolated enzymes is not necessarily the same as that on enzymes in cells. The associated colloids dominate the action of enzymes, and factors of the environment may act chiefly in the liberation of enzymes from the associated colloids or vice versa in more completely adsorbing the enzymes on their associated colloidal substances.

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A POSSIBLE NON-ENZYMATIC MECHANISM OF CHANGES OCCURRING IN THE PECTIC SUBSTANCES AND OTHER POLYSACCHARIDES IN LIVING PLANTS[1]

Z. I. Kertesz

It is usually assumed that enzymes are responsible for most chemical changes occurring in plant tissues. Yet, occasionally such reactions take place without the apparent presence of the corresponding enzymes.

The ripening of many fruits, as apples (**4**), pears, peaches (**1**), and tomatoes (**2**), for instance, is closely associated with progressive transformations of their pectic constituents. These reactions have been commonly assumed to be the results of enzymatic activity. Considerable effort to find the enzymes which cause the hydrolysis of polygalacturonic acid has failed (**6**), as has a more recent attempt to show their presence in apples (**3**). The purpose of this note is to suggest a non-enzymatic reaction which is likely to occur in plants and which may be responsible for some of the changes which occur in pectic substances as well as in other polysaccharides.

It has been known for some time that strong oxidizing agents hydrolyze polysaccharides and transform the component sugars into the corresponding dicarboxylic acids. On the other hand, dilute hydrogen peroxide acts on starch to yield dextrins and maltose (**5**). This observation was recently reiterated by Robertson, Ropes, and Bauer (**8**) who state that both starch and pectin are degenerated by hydrogen peroxide in the presence of ascorbic acid.

The effect of hydrogen peroxide on the pectic substances and the transformations which are caused will be dealt with elsewhere. Suffice it to say here that dilute solutions of hydrogen peroxide rapidly degenerate dissolved pectin even in the absence of ascorbic acid. The pectin loses its typical colloidal properties, becomes soluble in 70 per cent. alcohol and is transformed into a material which may not be classified any longer as a pectic substance. It is likely that this reaction with hydrogen peroxide is not typical for pectin and starch alone but that it will also be observed with other polysaccharides.

It is suggested that some of the changes in the polysaccharides and pectic constituents of plants *in situ* may be the result of the action of peroxides on these substances. The presence of peroxides in plants has never been conclusively demonstrated. It has been shown, however, that a number of dehydrogenase systems are capable of forming hydrogen peroxide *in vitro*, at least, and that many reactions occur in plants which necessitate the assumption of the presence of peroxides in the tissue. It has been often suggested that hydrogen peroxide or organic peroxides are cell constituents and that their absence, as shown by chemical tests, is only indicative of their transient character.

[1] Journal Paper no. 534, New York State Agricultural Experiment Station, Geneva, N. Y. Approved for publication on November 16, 1942.

Now, if peroxides are present in the plant tissue, it may well be that one of their functions is the degeneration and decomposition of starch and pectin. This reaction may also be responsible for the transformation of protopectin into soluble pectin (pectinic acid). It is likely that protopectin is but a pectinic acid of much higher molecular or micellar weight (**7**). Its transformation into soluble pectin would be essentially the same reaction as the subsequent degradation of pectin (pectinic acid) into d-galacturonic acid. Thus the effect of peroxide may, theoretically at least, replace the function of both protopectinase and pectinase (pectin-polygalacturonase).

If hydrogen peroxide or organic peroxides can be shown to be performing transformations of polysaccharides in plants, this would open up further avenues of speculation concerning the interrelation between oxido-reduction reactions and carbohydrate changes. It is of special interest to note in this connection that hydrogen peroxide apparently occurs among the reaction products during the non-enzymatic decomposition of ascorbic acid (**9**).

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AN AIR PUMP OF LOW CAPACITY[1]

EARL B. WORKING

(WITH TWO FIGURES)

For a study of the respiration and heat production of wheat it was desired to draw a slow stream of air through the grain over long periods of time. Too rapid aspiration greatly increased the difficulty of holding the moisture content of the grain constant, but at slow rates, needle valves or rubber tubing constricted with screw clamps were difficult to adjust accurately and were likely to clog up during the night and void the experiment.

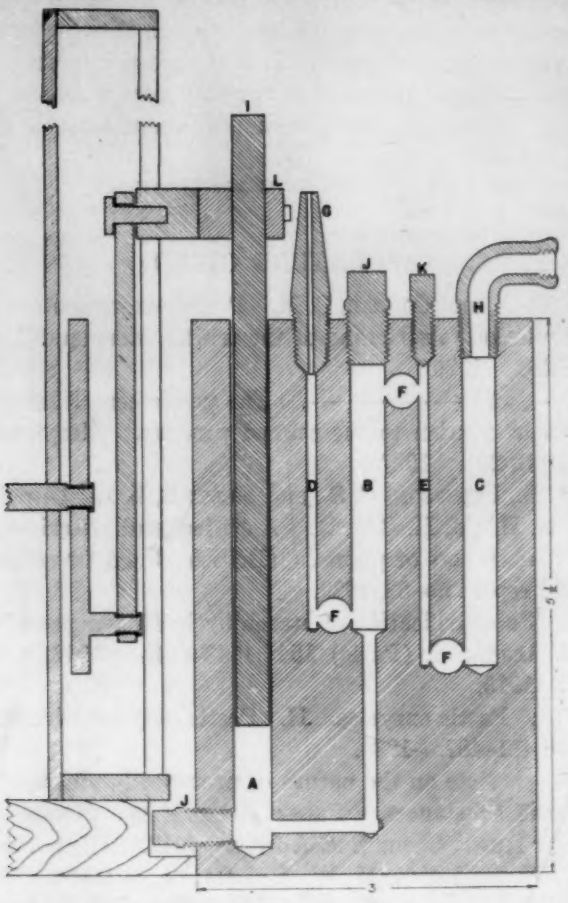

FIG. 1. Air pump drilled from aluminum alloy.

An adjustable pump of low capacity seemed to offer the best solution. It was decided to use mercury valves, since very little leakage or sticking in other types of valves might cause complete failure to pump. It was believed that a mercury piston would remove the possibility of leakage at that point.

[1] Contribution no. 93 Department of Milling Industry.

In the lubrication of motorcycle motors the principle has been used that the output of a pump can be varied continuously from zero to maximum without change of the rate or length of stroke, if the position of the piston is adjustable so that any desired portion of the stroke may occur before the piston reaches the inlet port, and the inlet valve consists only of the closing of the inlet port by the passage of the piston. This principle of adjustment is especially easy to apply to a mercury piston.

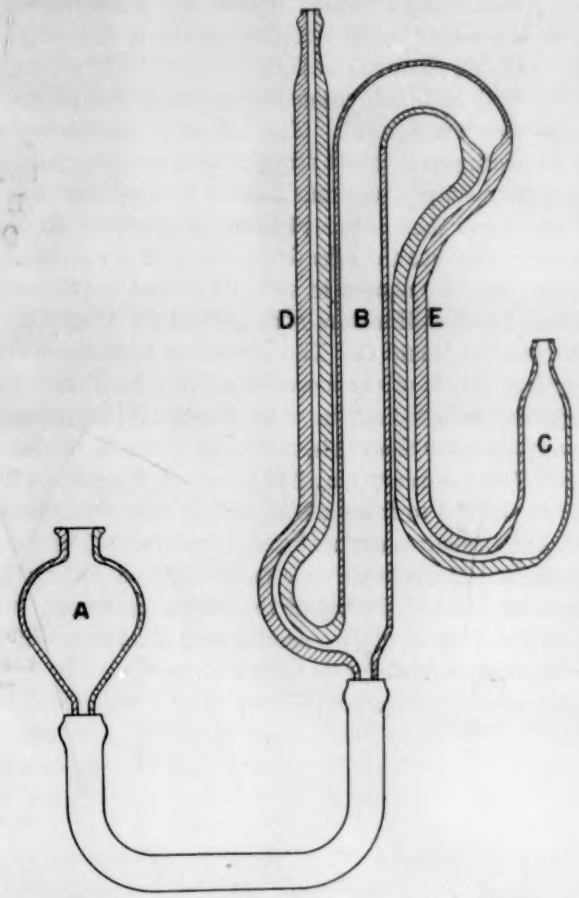

FIG. 2. Air pump constructed of glass and rubber tubing.

The first of these pumps was drilled out of a bar of hard aluminum alloy, $1 \times 3 \times 5\frac{1}{2}$ inches, as shown in figure 1, because it was to be used in a location where there was serious danger of breakage of a glass pump. The connections, F, F, F, between the vertical bores, D, B, E, and C, were made by drilling in from the side, and closing the holes with $\frac{1}{8}$-inch iron pipe plugs, such as were also used at J, J. Because of space limitations, a special plug, K, was made from $\frac{1}{4}$-inch rod to close the upper end of bore E.

For operation, bore A is filled with mercury to a level immediately below the opening of B into D, and barely enough mercury is put into C to fill bore

E when there is sufficient suction on that side. The steel plunger, I, is adjustable vertically in clamp L so that at the bottom of its stroke it may displace any amount from zero to nearly all of the mercury in the reservoir A. As this mercury is displaced, it rises in B, first closing the inlet valve from D, and then forming the piston of the pump, forcing air from B through the small amount of mercury forming a seal at the bottom of C. On the return stroke, however, this seal will withstand a suction of about 2½ inches of mercury because of the small volume of bore E. Thus the suction or pressure obtainable from such a pump is limited chiefly by the length of the small diameter drill available to cut D and E.

Ordinarily it will be much easier to construct the pump of glass and rubber tubing as shown in figure 2. This also obviates the necessity of using guides and a connecting rod to get straight line reciprocating motion, since the reservoir A may simply be hung from a slowly-revolving crank. The output of the pump can then be varied from maximum to zero by raising the body of the pump to reduce the effective portion of the stroke. The dimensions of the pump may be chosen to suit individual requirements. A bore diameter of from 1 to 2 millimeters is suggested for D and E, and of from 10 to 20 millimeters for B and C. The suction or pressure obtainable is limited by the vertical height of the mercury column in E, and 10 to 15 centimeters is suggested as sufficient in most cases. If the pump is operated against a pressure greater than this, as would occur if the inlet or exhaust were left closed off, the mercury may be blown out of capillary E, so the tube B is bent over at the top to form a trap so that the mercury will run back down into E when pressures are restored to normal. The pump would be slightly more efficient if the bend were made in the capillary E, so that the mercury piston could rise to the top of the larger diameter tube B, in which case it would be desirable to place a small trap near the top of E to prevent the loss of the mercury which forms the valve.

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NOTES

Sectional News.—The editor wishes to remind the chairmen and secretaries of all sections, both regional and local, that PLANT PHYSIOLOGY desires to disseminate sectional news to the membership at large. It is necessary, however, that the news be sent in voluntarily, and in time for appropriate use. Announcements must be sent in long enough before the events to have them reach the members ahead of the events. Spring meetings cannot be announced any later than the April number; and copy must be in hand some weeks before the journal appears, or it cannot be used. Reports of meetings are desirable, but should be sent in promptly after the meetings, so that the reports are timely. It is not possible to write to the officers of sections for such information, and the journal will carry only such notices as are sent in early enough to meet the suggested timing.

Manuscripts.—It seems desirable once more to call attention to the machinery set up for the handling of manuscripts offered to PLANT PHYSIOLOGY. The editor-in-chief no longer circulates papers to the readers. That function is performed by the secretary of the editorial committee, Dr. WALTER F. LOEHWING, Department of Botany, The University of Iowa, Iowa City, Iowa. When papers are sent direct to Chicago, they do not receive attention for some weeks. There is no time for the readdressing and reshipment of such manuscripts. They accumulate in a drawer until they get in the road; then they may receive attention, when a single trip will care for numbers of them. If authors wish prompt action on their papers, they can get it, by following the rules which are published on the third cover page of every number of the official journal. There is enough work on the actual editorial work that we cannot spend time on the manuscripts which are entered through improper channels. We want to be helpful, prompt, courteous to all; but we need the cooperation of all writers who desire to publish in PLANT PHYSIOLOGY. Your journals can reach you more promptly if we do not have so many extraneous errands to run.

Membership List.—The secretary is preparing a new membership list. It has always seemed to appear at the wrong time of year, so the editor wishes to make a suggestion. There is one period when the membership list is necessary to intelligent action; that is the period of nominations and election of officers. When the list appears after that period is past, it is out of date by the time another election comes round. Would it not be much better to prepare the membership list during the autumn, or late summer, and send it out either with the October or January number of PLANT PHYSIOLOGY? It would then be in hand for the intelligent selection of nominees, and the mailing with the journal should save some postage. Some other societies publish

their roster of members with their annual year-books. We could add the membership list following the index in the October number of PLANT PHYSIOLOGY, and so provide a solution to a problem which has long wanted intelligent action.

Enzymology.—The third volume of this series, *Advances in Enzymology and Related Subjects of Biochemistry*, has been published by Interscience Publishers, Inc., 215 Fourth Ave., New York City. The series is edited by F. F. NORD, of Fordham University, and C. H. WERKMAN, of Iowa State College. All plant physiologists will be grateful that it bears the portrait of RICHARD WILLSTÄTTER as a frontispiece, in memory of this great biochemist who died in 1942 at the age of 70. There are eleven summaries of progress in the field of enzyme chemistry. These are entitled as follows: Chromosomes and nucleoproteins, by A. E. MIRSKY; effects of temperature on enzyme kinetics, by IRWIN W. SIZER; x-rays and the stoichiometry of the proteins, by W. T. ASBURY; the chemistry of glycogen, by KURT H. MEYER; verdoperoxidase, by KJELL AGNER; mechanisms of carbohydrate metabolism, by E. S. GUZMAN BARRON; the intermediary stages in the biological oxidation of carbohydrate, by H. A. KREBS; the chemistry and biochemistry of pantothenic acid, by ROGER J. WILLIAMS; the chemistry and biochemistry of biotin, by KLAUS HOFMANN; recent progress in tumor enzymology, by JESSE P. GREENSTEIN; and the rôle of microorganisms and enzymes in wine making, by W. V. CRUESS.

The standing of the various writers in their field guarantees that the summaries of progress are well considered reports of the work recently accomplished in enzymology and closely related biochemical research. In addition, this volume has not only an author and subject index, but it contains a cumulative index for the three volumes which have thus far appeared in the enzymology series. This cumulative index is very short, but lists the main things, authors, and subjects in broad terms, so that all three volumes may be consulted quickly for any contributor's review, and for the general subject. It makes use very easy.

The publishers, contributors, and the editors deserve our thanks and congratulations that so much service can be rendered to their fellow workers during these troublous times. The price is only $5.50 per copy, which easily puts it in reach of all workers and libraries. It is highly commended, and will no doubt receive a hearty welcome from all workers in this field of investigation.